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Effect of extraction buffers on characterization of isoenzymes from vegetative tissues of five conifer species: A user's manual

J.A. Pitel and W.M. Cheliak

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PNFI

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Every five years, the FSSB publishes Canada's Forest Inventory; the official report on the location, extent, species, and condition of the forest resource. In addition, the FSSB is working closely with the provinces to expand the information available on changes to the forest from fire, harvesting, insects and disease, and from forest management activities. This information is essential to the development of sound policies for the improved management of this important and renewable natural resource.

EFFECT OF EXTRACTION BUFFERS ON CHARACTERIZATION OF ISOENZYMES FROM VEGETATIVE TISSUES OF FIVE CONIFER SPECIES: A USER'S MANUAL

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J.A. Pitel and W.M. Cheliak

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Abstract

Methods to improve the analysis of isoenzymes by starch gel electrophoresis of vegetative tissues of five conifers were investigated. A control buffer without any protective agents, together with 15 existing or modified extraction buffers containing a variety of protective agents, were tested on extracts prepared from bud, bark, and needle tissues of jack pine, Scots pine, white spruce, black spruce, and tamarack to ascertain the best extraction buffer for each enzyme, tissue, and species. The results indicated that few, if any, enzyme protective chemicals are needed for buds with scales removed. However, for needle and bark tissues, protective agents were essential to obtain good activity and resolution of the enzymes. Variations in the types and combinations of protective agents in the different extraction buffers affected the total enzyme activity, resolution, and number of bands of activity in the electrophoretic patterns obtained. Tables are presented outlining those extraction buffers which produced the best activity and resolution for 41 enzymes extracted from bud, bark, and needle tissues for each of the five conifers. The Tables also indicate the enzymes potentially useful for further genetic studies.

Résumé

On a étudié des moyens de perfectionner l'analyse, par électrophorèse en gel d'amidon, des isoenzymes des tissus végétatifs (bourgeons, écorce et aiguilles) de cinq conifères (pin gris, pin sylvestre, épinette blanche, épinette noire et mélèze laricin). On a expérimenté sur des extraits de ces tissus une solution tampon témoin, exempte d'agents de protection, ainsi que 15 solutions tampons modifiées ou non et contenant divers agents de protection, fin de déterminer laquelle était la plus efficace à l'égard de chaque enzyme, de chaque tissu et de chaque essence. Les résultats montrent que peu ou point d'agents chimiques protecteurs des enzymes sont nécessaires pour les bourgeons écaillés. Toutefois, pour les aiguilles et l'écorce, ces agents sont essentiels pour obtenir une bonne activité et une bonne résolution des enzymes. Les types et les combinaisons variables d'agents protecteurs, dans les différentes solutions tampons, ont influé sur l'activitié, la résolution et le nombre de bandes d'activité des enzymes, dans leur ensemble, sur le diagramme électrophorétique. Des tableaux présentent les meilleures solutions tampons pour 41 enzymes extraites des tissus des bourgeons, de l'écorce et des aiguilles de chaque conifère. Les tableaux désignent aussi les enzymes qui pourraient ultérieurement servir à des études de génétique.

EFFECT OF EXTRACTION BUFFERS ON CHARACTERIZATION OF ISOENZYMES FROM VEGETATIVE TISSUES OF FIVE CONIFER SPECIES: A USER'S MANUAL

INTRODUCTION

Isoenzymes provide convenient genetic markers to study the amount, distribution, and maintenance of genetic variability in natural and artificial populations (Feret and Bergmann 1976, Nevo 1978, Brown 1979, Conkle 1981, Cheliak and Pitel 1984b). Forest trees, conifers in particular, can provide many sources of tissue from which to make crude protein extracts for subsequent electrophoresis. Seed tissue of conifers has often been preferred because it is easily stored, has few secondary metabolites to interfere with enzyme activity, and the genetic control of electrophoretic variants can be inferred and tested directly.

However, despite these advantages, there are instances when it is more desirable to analyze isoenzymes extracted from vegetative tissues. For one thing, most vegetative tissues are available during years of poor seed production. For another, tissue samples can be obtained from juvenile as well as sexually mature trees, they can be collected and kept in a frozen state until needed, and logistical problems of sampling during a short period of time can be avoided. As well, inference of diploid genotypes from haploid tissues is inefficient, compared to direct observation from somatic tissue.

Extracting enzymes and maintaining them with good activity and resolution from vegetative tissues presents numerous problems due to the presence of phenols, quinones, and other components. Such substances may inhibit or precipitate enzymes (Loomis and Battaile 1966, Anderson 1968, Loomis 1974, Haissig and Schipper 1978). A few reports are available describing some success in extracting isoenzymes from vegetative tissues of conifers (Kelley and Adams 1977, Mitton et al. 1979). However, it is not expected that a single extraction buffer will be suitable for all enzymes of the various species and tissues involved (Feret and Bergmann 1976). One objective of this preliminary study was to test various existing and modified extraction buffers on their ability to maintain good activity of enzymes from three tissues of each of the five species of trees. A further objective was to maintain a rapid and simple method of analysis that would permit the screening of large numbers of samples from as wide a range of enzymes as possible.

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Organization of the Report

This report describes results of the starch gel electrophoresis of about 40 enzymes of crude extracts from buds, bark, and needles for each of the five conifer species. It is intended to relate to the reader some of our experience in working with vegetative tissues of conifers. No one extraction buffer will be suitable for all species and tissues. We thus have tried several extraction buffers present in the literature, as well as others that we have formulated ourselves (Table 3) in order to determine those best for each particular tissue and species. Although, of course, numerous other buffer combinations are possible, we have achieved sufficiently good results so far with those that we have tried. We successfully utilized extraction buffer no. 5 in a recent genetics experiment (see Cheliak and Pitel 1984b).

The 'Results' are divided into five principal sections, each covering a different species of tree. Within each section, each paragraph describes the observations of one enzyme for each of three different tissues in terms of R_fs for each band, the type of resolution, and the intensity of activity observed. The enzymes are described in alphabetical order. For example, the first paragraph describes the results for acid phosphatase (ACP). ACP is followed by aconitase (ACO) and so on.

The results are summarized in tabular form (Tables 4-23) that describe, for each tissue and species, the extraction buffer(s) that proved best for each enzyme, the type of activity and resolution obtained, as well as the number of bands observed for each enzyme for the 16 different extraction buffers. Table 24 lists those buffers which were successful for most enzymes for a particular tissue and species.

A word of caution is in order. Please note that different R_f values and numbers of bands may be obtained if different gel and electrode buffers are used. Also, other staining recipes, such as those using other substrates for PER besides benzidine, may reveal some minor differences in banding patterns. We also note that certain enzymes such as CTO, PER, and PPO, may possess similar bands with respect to their R_f values. Due to these difficulties the staining method for each eznyme used in this study is presented in detail elsewhere (Cheliak and Pitel 1984a).

MATERIALS AND METHODS

Bud, bark, and needle tissues were collected from single trees of jack pine (Pinus banksiana Lamb.), Scots pine (Pinus sylvestris L.), white spruce (Picea glauca [Moench] Voss), black spruce (Picea mariana [Mill.] B.S.P.), and tamarack (Larix laricina [Du Roi] K. Koch.). Samples were collected from December to February except for larch needles, which were collected in June. The bark and needle tissues obtained were of recent years' growth. Samples were stored in heavy mill plastic bags at -20°C until needed. All trees were located at the Petawawa National Forestry Institute, Chalk River, Ontario, Canada (lat. 45°59'N and long. 77°21'W). Samples of bark and needle tissues, 100 mg in size, were ground with sand, using a mortar and pestle. Volumes of extraction buffer used were 0.8 and 0.5 ml for bark and needles, respectively. For buds (with scales removed) a 10 mg sample with 0.06 ml of extraction buffer was placed in a 0.5 ml conical, polystyrene sample cup (Elkay Products Inc., Shrewsbury, MA) and homogenized with a motorized teflon grinding head. Duplicate extractions were made with each of the 16 extraction buffers listed in Table 3. Buffers were prepared fresh each week.

The liquid extracts were absorbed on 2X12.5 mm paper wicks cut from Whatman No. 3MM filter paper and immediately inserted into 12.5% (W/V) starch gels which were prepared from Electrostarch (Electrostarch Co., Madison, WI, U.S.A.; lot No. 392) and Connaught starch (Connaught Laboratories, Willowdale, Ontario, Canada) in a 1:1 ratio. The composition of the gel and electrode buffers and the power used are listed in Table 2. The enzymes resolved in each buffer system are shown in Table 1. Each gel contained samples of all 3 tissues from the same tree. Electrophoresis of the various extracts was repeated at least once. After the buffer front had migrated a total of 6 cm the gels were removed and sliced horizontally into 1 mm sections. The staining recipes for the 41 enzymes were essentially as described by Shaw and Prasad (1970), Hamaker and Snyder (1973), Siciliano and Shaw (1976), Yeh and O'Malley (1980) and Cheliak and Pitel (1984a). Diagrams were made of the isoenzyme banding patterns and included staining intensities and estimates of the relative migration distance for each band. The rating "poor" indicates unsuitability for scoring, whereas the ratings "satisfactory" and "good" are suitable for this purpose.

RESULTS

Jack Pine

When analyzed by the gel and electrode buffer system "H" (Table 2), a total of six isoenzyme bands for ACP were observed for both bud and bark tissue extracts. The R_fs were 0.12, 0.19, 0.27, 0.34, 0.42, and 0.46. For needle extracts the band of activity with R_f 0.46 was not observed. As indicated in Tables 5-7, the number of bands for each tissue varied with the extraction buffer used (Table 3). Only three bands of activity were observed for all tissues when the "A" buffer system was used. The bands did not migrate as fast as for the "H" buffer system. The recommended extraction buffers to use for each tissue are listed in Table 4.

Only one weak band of activity of ACO, with R_f 0.30, was observed for four needle extracts (Tables 4,5). Two bands of ACO activity with R_fs of 0.30 and 0.38 were detected for extracts from bark tissue (Table 6). The type of extraction buffer used affected the level of enzyme activity obtained. Only extraction buffers 5, 6, 7, 12, and 14 produced satisfactory results (Table 4). Two bands of activity were also observed for bud extracts (Table 7). However, unlike bark and needle tissues, good levels of activity were observed for most of the 16 bud extracts (Table 4).

Needle and bark extracts both resulted in poor activity and resolution for AK (Table 4). For many extracts, no enzyme activity was observed (Tables 5,6). For extracts from buds, four bands with R_fs of 0.13, 0.24, 0.32, and 0.39 were observed (Table 7). Satisfactory results were obtained using four of the 16 extraction buffers as shown in Table 4.

Two bands of activity with R_fs of 0.37 and 0.39 were observed for ADH obtained from needles. While many extracts displayed no activity, satisfactory results were only obtained with extraction buffers 3 and 12 (Tables 4,5). Two bands of activity were also observed for bark tissue. Five extraction buffers produced satisfactory results (Table 4). For buds, two bands of activity were present for most extracts, although some extraction buffers were not very effective in producing satisfactory activity for ADH (Tables 4,7).

Only one band of activity for ALD of R_f 0.30 was observed for needle extracts (Table 5). Satisfactory results could be obtained with several of the different extraction buffers (Table 4). A total of three isoenzyme bands with R_fs of 0.09, 0.17, and 0.30 were observed for some bark extracts (Table 6).

Good ALD activity was observed for all extracts of bud tissues (Table 4). Four bands of activity were present with R_fs of 0.09, 0.17, 0.30, and 0.33.

Five bands of activity with R_fs of 0.09, 0.16, 0.19, 0.30, and 0.41 were observed for ALP obtained from needles. Best activity was obtained when needles were extracted with buffers 8 through 12 (Tables 3-5). Five bands of activity were also observed for bark extracts. All extraction buffers gave similar results (Tables 4,6). For buds, some extraction buffers were more effective than others in maintaining satisfactory levels of enzyme activity (Table 4).

Needle extracts analyzed for AAT contained from one to four bands of activity depending on which extraction buffer was used (Table 5). Only extraction with buffer 3 resulted in 4 bands. The R_fs were 0.06, 0.40, 0.46, and 0.53. The activity and number of isoenzyme bands obtained from bark tissue also varied with the type of extraction buffer used (Tables 4,6). For buds, extraction with buffer 6 resulted in a fifth isoenzyme band being detected at R_f 0.43 (Tables 3,7).

Only one band of CAT activity with an R_f of 0.09 was detected for both needle and bark tissues. Also, for each tissue, the activity was similar among the different extracts (Tables 4-6). A second band of activity with an R_f of 0.06 was observed for all bud extracts (Table 7).

Needle extracts analyzed for CTO all contained nine isoenzyme bands of activity with R_fs 0.18, 0.23, 0.33, 0.39, 0.49, 0.54, 0.59, 0.63, and 0.73 (Table 5). Good activity was present for most extracts (Table 4). Although 9 bands of activity were also observed for most bark extracts, the activity was not as good as for needle tissue (Table 4,6). Enzyme activity was poor for all bud extracts (Table 4). Also, two isoenzyme bands with R_fs 0.49 and 0.63 were not present (Table 7).

Six isoenzyme bands of DIA activity with R_fs of 0.28, 0.34, 0.56, 0.60, 0.64, and 0.67 were observed for needle extracts in which extraction buffers 3, 10, 11, and 12 were used. Fewer bands were observed in all of the other extracts (Table 5). However, activity was poor for all extracts (Table 4). The band of activity with R_f of 0.56 was not detected in bark extracts. Activity was satisfactory when the bark tissue was extracted with buffers 2 through 6. A total of nine bands of activity were observed with several bud extracts (Table 7). The three

additional bands of activity not observed for needle tissues had R_fs of 0.38, 0.41, and 0.45.

When analyzed with the electrophoresis buffer system "H" (Table 2), a total of four isoenzyme bands of EST were observed for needle extracts. The R_fs were 0.17, 0.21, 0.25, and 0.32. Some extracts had fewer than four bands (Table 5). Bark tissue extracts contained four bands, while for bud extracts only two bands were present (Tables 6,7). When analyzed by the electrophoresis buffer system "B" (Table 2), a total of eight bands were observed for all three tissues. However, resolution was poor.

Needle, bark and bud extracts analyzed for F-EST all contained two bands of activity with R_fs 0.23 and 0.80 (Tables 5-7). The band with R_f 0.23 was the most active for all three tissues.

No activity was observed for β-GAL with either needle or bark extracts. One band of activity with R_f 0.18 was detected for all 16 bud extracts (Tables 4,7).

Two isoenzyme bands of β-GLU with R_fs of 0.21 and 0.52 were observed for all bud and bark extracts, while only one band (with R_f 0.52) was present for needle extracts (Tables 5-7).

Three isoenzyme bands of GLUDH with R_fs of 0.36, 0.38, and 0.40 were observed for needle extracts in which extraction buffers 2, 3, 11, and 12 were employed. No activity was observed for most of the other extracts (Table 5). Only one band of activity with R_f 0.36 was observed for bark extracts, while a fourth isoenzyme band with R_f 0.19 was detected for extracts 11 and 12 from bud tissues (Tables 6,7).

Two isoenzyme bands of G6PD with R_fs 0.27 and 0.35 were observed for most bud and bark extracts (Tables 6,7). Only one band of activity with R_f 0.27 was detected for needle extracts (Table 5). Satisfactory activity was obtained with several of the 16 extraction buffers (Table 4).

A single band of GDH with R_f 0.25 was observed for bud, needle, and bud extracts (Tables 5-7). Good activity was obtained for 6 needle extracts and most bud extracts (Table 4).

Two isoenzyme bands of GPT with R_fs 0.22 and 0.55 were observed for all bud and bark extracts (Tables 6,7). Very high activity was present for the band of activity with R_f 0.55 for most of the bud extracts. One band (with R_f 0.22) of poor activity was detected with all needle extracts (Table 5).

For most bud extracts, three bands of G_3PDH with R_fs 0.12, 0.19, and 0.28 were detected (Table 7). However, only two bands (with R_fs 0.19 and 0.28) were observed with needle extracts, and only one band (with R_f 0.28) was observed for bark extracts (Tables 5,6). Satisfactory activity was present only for several bud extracts (Table 4).

Two isoenzyme bands of α-GPDH with R_fs 0.19 and 0.25 were observed for bark and needle tissue extracts (Tables 5,6). Although two bands were also detected for bud extracts, the band of activity with R_f 0.19 was not present, while a band of activity with R_f 0.16 was observed. Enzyme activity was poor for all three tissues (Table 4).

No activity of G_2DH was observed for extracts of buds and bark (Tables 6,7). However, one band of activity with R_f of 0.18 was observed for most needle extracts (Table 5). Good activity was obtained for eight of the 16 extracts (Table 4). Electrophoresis buffer "C" was used in this analysis (Table 2).

Three isoenzyme bands of HK with R_fs 0.24, 0.35, and 0.45 were observed for all bud extracts (Table 7). Only one band with R_f 0.35 was occasionally detected for needle and bark tissues. However, enzyme activity was not detected for most extracts (Tables 5,6).

One band of IDH activity with R_f 0.30 was observed for most of the 16 needle extracts (Table 5). As shown in Table 4, only a few extraction buffers were effective in maintaining satisfactory activity of IDH extracted from needles. Two bands of IDH, with R_fs 0.30 and 0.32, were observed for some bark extracts (Table 6). Although two bands of activity were detected for bud extracts, the band with R_f 0.32 was not present, while a band with R_f 0.23 was detected in some extracts (Table 7).

A total of three isoenzyme bands of LAP with R_fs 0.56, 0.70, and 0.74 were observed for most bud and bark extracts (Tables 6,7). Only two bands with R_fs 0.56 and 0.70 were detected for needle tissue (Table 5).

Four isoenzyme bands of MDH with R_fs of 0.10, 0.30, 0.35, and 0.40 were observed for bud extracts (Tables 4,7). Four bands were also present for some bark extracts. However, the band with R_f 0.44 was not detected in any of the needle extracts (Tables 5,6).

A total of four bands of ME with R_fs of 0.19, 0.22, 0.26, and 0.29 were observed for bud extracts. Only one band with R_f 0.26 was observed for both needle and bark extracts (Tables 5-7). As indicated in Table 4, only a few extraction buffers were effective in maintaining satisfactory enzyme activity. Some of the zones of activity observed on gels stained for ME may be actually MDH.

Three isoenzyme bands of MPI with R_fs of 0.26, 0.34, and 0.66 were observed for bud extracts. Activity was satisfactory only for a few extracts (Table 4). No activity was observed for needle extracts; while one band of very weak activity with R_f 0.26 was detected for some of the bark extracts (Tables 4-6).

Good activity of MR was observed for bud extracts (Table 4). For most extracts five bands with R_fs of 0.30, 0.33, 0.38, 0.43, and 0.46 were detected (Table 7). Enzyme activity was weaker for needle and bark extracts (Table 4). Also, the band of activity with R_f 0.30 was not observed for either tissue (Tables 5,6).

Enzyme activity of NADHDH was not detected for bark extracts (Tables 4,6). Three bands of activity with R_fs of 0.22, 0,43, and 0.66 were present for bud extracts (Table 7). For needle extracts, the band with R_f 0.43 was not observed, while a band with R_f 0.27 was present for many extracts (Table 5).

Two bands of NADPHDH with R_fs of 0.31 and 0.63 were detected for both bud and needle extracts (Tables 5,7). One band of R_f 0.63 with very weak activity was observed for several extracts from bark tissues (Tables 4,6).

Six isoenzyme bands of PEP with R_fs of 0.28, 0.38, 0.52, 0.56, 0.62, and 0.66 were observed for many bud and bark extracts (Tables 6,7). The bands of activity with R_fs of 0.52 and 0.62 were not detected for any of the needle extracts (Table 5).

Good activity of PER was observed for most needle and bark extracts (Table 4). For both of these tissues, isoenzyme bands with R_fs of 0.18, 0.23, 0.33, 0.39, 0.49, 0.54, 0.59, 0.63, and

0.73 were detected (Tables 5,6). Enzyme activity for buds was weaker (Table 4). Also, bands with R_fs of 0.49 and 0.68 were not detected (Table 7).

One band of strong PC activity with R_f 0.33 was observed for all bud extracts (Table 7). One band of activity with a similar R_f was detected with needle and bark extracts. However, for the few extracts in which a band was present, activity was poor for needles and bark (Tables 4-6).

Enzyme activity of 6PGD was good for most bud extracts (Table 4). Two bands with R_fs of 0.31 and 0.41 were detected (Table 7). Two bands of activity with similar R_fs were also observed for needle and bark extracts. However, satisfactory activity was present only for a few of the 16 extracts (Tables 4-6).

Three isoenzyme bands of PGI with R_fs of 0.40, 0.45, and 0.51 were observed for most bud and needle extracts. For bark extracts, two additional bands with R_fs of 0.53 and 0.57 were detected. Activity was good for all three tissues (Table 4).

Although activity of PGM was good for most of the bud, needle, and bark extracts, resolution was only poor or satisfactory (Table 4). A total of five isoenzyme bands with R_fs 0f 0.28, 0.30, 0.32, 0.46, and 0.52 were observed for most bud extracts. Needle extracts produced two bands with R_fs 0.30 and 0.52, while extracts from bark produced an additional band with R_f 0.46. The three cathodal bands may actually be zones of IDH activity.

Activity of isoenzymes of PPO was satisfactory for most bud, needle, and bark extracts (Table 4). The banding patterns observed were similar to that of PER, except for some variations in the number of bands detected (Tables 5-7).

Three isoenzyme bands of SDH with R_fs of 0.31, 0.35, and 0.39 were observed for bud and bark extracts (Tables 6,7). The band of activity with R_f 0.35 contained most of the activity. Only one band with R_f 0.35 was present for needles. The extraction buffers that resulted in the best activity are shown in Table 4.

Two bands of SUDH with R_fs of 0.13 and 0.21 were detected for most bud extracts. Activity and resolution were satisfactory for most extracts (Table 4). Only one band of activity with R_f

0.30 was present for bark and needle tissues. However, for many extracts from bark and needle tissues, no enzyme activity was detected (Tables 5,6).

Activity of SOD was good for most bud, needle, and bark extracts (Table 4). Five bands of activity with R_fs of 0.34, 0.42, 0.49, 0.70, and 0.80 were observed for needle and bark tissues. For bud tissue, the band of activity with R_f 0.34 was not present.

Scots Pine

When analyzed with the electrophoresis buffer system "B" (Table 2), 11 isoenzyme bands of ACP with R_fs of 0.14, 0.17, 0.20, 0.28, 0.31, 0.37, 0.41, 0.52, 0.57, 0.69, and 0.74 were observed for several extracts prepared from bark tissue. For extracts prepared from the other two tissues, some bands were not present (Table 10). For needle tissue, the band of activity with R_f 0.37 was not detected, while for bud tissue the bands with R_fs 0.14, 0.37, and 0.41 were not observed. Enzyme activity was best with bud tissue (Table 8). When analyzed by electrophoresis buffer system "A" (Table 2), fewer ACP bands were observed.

Two bands of AK activity, with R_fs of 0.14 and 0.32, were detected for bud and needle tissue (Tables 9,11). For extracts prepared from bark tissues, only one weakly staining band with R_f 0.32 was observed (Table 10). Activity was best with bud tissue (Table 8).

Although needle and bark tissues indicated two bands of ADH activity with R_fs 0.33 and 0.51, many extracts produced only one band, or no activity (Tables 9,10). Three bands of activity, with R_fs 0.33, 0.42, and 0.51, were observed for extracts of bud tissue. The type of extraction buffer used was extremely important to maintain suitable enzyme activity (Table 8).

When analyzed with electrophoresis buffer system "A" (Table 2), two bands of ALD activity with R_fs of 0.14 and 0.21 were detected for all 16 bud extracts and most bark extracts (Tables 10,11). For needles, only one band with R_f 0.21 was detected (Table 9). The extraction buffers that were most effective in maintaining enzyme activity are shown in Table 8.

Nine isoenzyme bands of AAT activity with R_fs of 0.08, 0.13, 0.18, 0.35, 0.38, 0.41, 0.57, 0.60, and 0.63 were observed for all bud extracts. Although nine bands of activity were also observed for needle and bark tissues, many extracts produced fewer bands (Tables 9,10).

Activity of CAT for bud tissue was poor, and only five extracts produced any enzyme activity (Tables 8,11). These bands of activity had R_fs of 0.11, 0.17, and 0.21. Four bands of activity with R_fs of 0.11, 0.17, 0.21, and 0.64 were observed for extracts prepared from needle and bark tissues (Tables 9,10).

A total of eight isoenzyme bands of DIA with R_fs of 0.33, 0.40, 0.46, 0.55, 0.66, 0.73, 0.76, and 0.95 were observed for most bud extracts (Table 11). Activity was consistently poor for both needle and bark tissues (Table 8). Three bands of activity with R_fs of 0.46, 0.66, and 0.73 were detected for many needle extracts. However, for bark tissues the band of activity with R_f 0.46 was not observed.

Three isoenzyme bands of F-EST activity with R_fs of 0.25, 0.48, and 0.71 were detected for all extracts prepared from needle, bark, and bud tissues. For buds, the band of activity with R_f 0.25 had the highest activity, while for needle and bark tissues, the band of activity with R_f 0.71 had the highest activity. Variations in activity were observed among the 16 different extracts (Table 8).

One band of β-GAL with R_f 0.18 was detected for all bud extracts. Activity was consistently good for all extracts (Table 8). No enzyme activity was observed for needle or bark tissues (Table 8).

Bud, needle, and bark tissues produced one band of β-GLU activity with R_f 0.45. However, good activity was present for several bud extracts (Table 8).

Two bands of GLUDH with R_fs 0.24 and 0.52 were observed for bud and bark tissues. The activity and number of bands present varied with the extraction buffer used (Tables 8,10,11). No activity was observed for extracts prepared from needle tissue.

Only one extraction buffer was effective in producing good activity of G6PD from needles (Table 8). One band of activity with R_f 0.25 was detected. For buds, two additional bands of activity with R_fs 0.14 and 0.18 were observed, while one additional band of activity with R_f 0.43 was detected for bark tissue.

One band of GDH with R_f 0.24 was observed for all bud and needle extracts and most bark extracts (Tables 9-11). Variations in enzyme activity were observed for the different tissues and extraction buffers used (Table 8).

Activity of GPT was not detected in bark tissue, while one band of activity with R_f 0.28 was observed for four needle extracts, and two bands of activity with R_fs 0.28 and 0.43 were

observed for all bud extracts (Tables 9-11). The extraction buffers that produced the best results are shown in Table 8.

Four isoenzyme bands of α-GPDH with R_fs of 0.17, 0.19, 0.29, and 0.32 were observed for extracts prepared from bud, needle and bark tissues. However, as shown in Tables 9-11, many extracts had fewer, or no bands present. Satisfactory activity and resolution was obtained using several extraction buffers listed in Table 8.

Satisfactory activity of G_2DH was present for most needle extracts (Table 8). Only one band of activity with R_f 0.20 was observed. Activity was consistently poor for both bud and bark extracts. For many extracts, no enzyme activity could be detected (Tables 10,11).

Four isoenzyme bands of HK with R_fs 0.10, 0.17, 0.30, and 0.32 were observed for most bud extracts (Table 11). Only one band of activity with R_f 0.32 was detected for needle and bark extracts. Activity was poor, or nonexistent, for most extracts of these two tissues.

One strong band of IDH activity with R_f of 0.24 was observed for most bud, needle, and bark tissues. Three minor bands of activity with R_fs of 0.11, 0.14, and 0.26 were also present for bud tissue. For needles, the band of activity with an R_f 0.26 was not detected, while for buds, the bands with R_fs of 0.11 and 0.14 were not present.

Two bands of LAP activity with R_fs of 0.61 and 0.71 were observed for bud, needle, and bark tissues. The level of enzyme activity observed varied with the extraction buffer used (Table 8).

Seven isoenzyme bands of MDH activity with R_fs of 0.09, 0.15, 0.18, 0.22, 0.26, 0.31, and 0.35 were observed for bud and needle extracts. For extracts from bark, the bands of activity with R_fs of 0.09, 0.15, and 0.18 were not detected. The activity and numbers of bands present varied with the extraction buffer (Tables 8-11).

Good enzyme activity and resolution of ME was obtained for extracts prepared from buds for many of the different extraction buffers (Table 8). Four bands of activity with R_fs of 0.18, 0.22, 0.26, and 0.30, were observed for bud tissue. For needles, three bands of activity with R_fs of 0.11, 0.18, and 0.26 were

observed, while for bark, three bands of activity with R_fs of 0.18, 0.22, and 0.26 were detected. Overlap of some bands with that of MDH was observed.

Three isoenzyme bands of MR with R_fs of 0.28, 0.34, and 0.40 were observed for bud tissue. Activity was good for several extracts (Table 8). Only one weakly staining band of MR activity with an R_f 0.40 was detected for both needle and bark tissue.

Good activity and resolution of NADHDH producing zones of activity with R_fs of 0.32, 0.37, 0.45, 0.60, 0.65, and 0.90 was observed for extracts from bud tissue (Tables 8,11). No enzyme activity was detected for extracts from needle or bark tissue.

Two bands of NADPHDH activity with R_fs of 0.34 and 0.60 were observed for extracts from bud and needle tissues. Activity was satisfactory for only a few needle extracts (Table 8). No enzyme activity was detected for bark tissue.

Activity of PEP was satisfactory for bud and needle tissues, but poor for bark tissue. Four isoenzyme bands with R_fs of 0.30, 0.46, 0.61, and 0.72 were observed for all three tissues (Tables 9-11).

Nine bands of PER activity with R_fs of 0.36, 0.40, 0.45, 0.51, 0.60, 0.67, 0.71, 0.75, and 0.77 were observed for extracts prepared from bud tissue. Five bands of activity with R_fs of 0.51, 0.53, 0.60, 0.67, 0.71, and 0.75 were observed for needle tissue, while five bands of activity with R_fs of 0.51, 0.60, 0.67, 0.75, and 0.77 were observed for bark tissue. The enzyme activity and resolution obtained for each tissue was similar for all extracts (Table 8). The electrophoretic mobilities of bands of CTO and PPO were very similar to that of PER for each tissue. However, the numbers of bands observed varied with the enzyme analyzed (Tables 9-11).

One band of PC activity with an R_f of 0.34 was observed for bud tissue. Activity was good for most extracts prepared from buds. Activity of PC for needles and bark was present only for a few extracts (Tables 8-11).

Two bands of 6PGD activity with R_fs of 0.29 and 0.32 were observed for extracts prepared from bud, needle, and bark tissues. However, the level of activity and the number of bands observed, varied with the type of extraction buffer used (Tables 8-11).

Activity of PGI was good for most bud and needle extracts (Table 8). Five bands of activity with R_fs of 0.36, 0.41, 0.46, 0.50, and 0.54 were detected with bud tissue, while for needles, the band of activity with R_f 0.41 was not present. Activity of PGI was satisfactory for most bark extracts. Five bands of activity with R_fs of 0.46, 0.50, 0.54, 0.60, and 0.65 were observed on gels stained for PGI.

Two major bands of PGM with R_fs of 0.65 and 0.75 were detected for bud, needle, and bark tissues. The activity and resolution obtained varied with the different extracts (Table 8). Three additional bands of lower activity with R_fs of 0.22, 0.24, and 0.26 were detected for extracts prepared from bud tissue, while for needles and bark, only the band with R_f 0.24 was observed. Overlap of the cathodal bands with that of IDH was observed.

Activity of SDH was best for extracts prepared from bud tissue. Three bands of activity with R_fs of 0.24, 0.31, and 0.40 were observed. Two bands with R_fs of 0.24 and 0.40 were detected for needles, while only one band with R_f 0.40 was detected for extracts from bark tissue (Tables 9,10).

Five bands of SUDH with R_fs of 0.15, 0.18, 0.21. 0.28, and 0.32 were observed for bud, needle and bark tissues. Activity was good for most bud extracts (Table 8). However, enzyme activity was low or not present for many needle and bark extracts (Tables 8-10).

Activity of SOD was good for all bud extracts. Three bands of activity with R_fs of 0.70, 0.77, and 0.82 were observed. Two bands of activity with R_fs 0.58 and 0.77 were detected for extracts prepared from needle and bark tissues. Activity was satisfactory for all extracts (Table 8).

White Spruce

When analyzed by the gel and electrode buffer "H" (Table 2), six isoenzyme bands of ACP activity with R_fs of 0.12, 0.15, 0.21, 0.27, 0.33, and 0.36 were observed for extracts prepared from bud, needle, and bark tissues. The number of bands and level of enzyme activity detected for each tissue varied with the type of extraction buffer used (Tables 12-15). The activity and resolution of ACP obtained using gel and electrode buffers A, B, or C were not as satisfactory.

Two isoenzyme bands of ACO activity with R_fs of 0.21 and 0.25, were observed for preparations from bud, needle and bark tissues. Activity was good for most bud extracts, while for most needle and bark extracts it was low (Table 12).

Activity and resolution of AK was satisfactory only for extracts from bud tissue (Table 12). Six bands of activity with R_fs of 0.14, 0.21, 0.29, 0.40, 0.55, and 0.68 were observed for buds, while only three weak and unresolved bands with R_fs 0.21, 0.29, and 0.40 were detected for needle and bark tissues.

Only one band of ADH activity with R_f 0.50 was observed for bud, needle, and bark tissue. However, for most needle extracts, no activity was detected (Table 13). The best ADH activity was observed for two bud extracts (Table 12).

Activity and resolution of ALD was satisfactory for all bud extracts and several needle and bark extracts (Table 12). Two bands of enzyme activity with R_fs of 0.05 and 0.15 were observed for buds. An additional band of activity with R_f 0.13 was observed for needles, while for bark extracts the third band of activity had an R_f of 0.09.

When analyzed using the gel and electrode buffer system "B" four bands of ALP activity with R_fs of 0.27, 0.33, 0.60, and 0.80 were observed for extracts from bud tissue. Resolution was poor near the origin of the gel. Only one band of ALP activity with R_f 0.80 was detected for needles, while two bands of activity with R_fs of 0.80 and 0.60 were observed for bark tissue.

Four bands of AAT activity with R_fs of 0.38, 0.48, 0.56, and 0.60 were observed for extracts from bud, needle, and bark tissues. Activity and resolution were good for all extracts prepared from buds. However, the type of extraction buffer used was important in obtaining satisfactory results for extracts from needle and bark tissues (Tables 12-14).

Only one band of CAT activity with R_f 0.26 was observed for all bark extracts, while two bands of activity with R_fs 0.15 and 0.26 were detected for all bud extracts. Activity was satisfactory for most extracts prepared from bud tissue (Table 12). No activity was detected for extracts prepared from needle tissue.

Activity of CTO was good for extracts prepared from needle and bark tissues, but poor for those from buds (Table 12). Four bands of activity with R_fs of 0.61, 0.66, 0.74, and 0.79 were detected for buds; seven bands of activity with R_fs 0.44, 0.47, 0.56, 0.61, 0.66, 0.83, and 0.90 were observed for needles; and nine bands of activity with R_fs of 0.44, 0.47, 0.50, 0.54, 0.56, 0.61, 0.66, 0.69, and 0.90 were detected for bark tissue.

Eight bands of DIA activity with R_fs of 0.18, 0.34, 0.46, 0.54, 0.59, 0.64, 0.69, and 0.95 were observed for extracts made from bud and needle tissues. For bark preparations, the bands with R_fs of 0.18 and 0.54 were not detected. Activity was good for all bud extracts (Table 12). Only a few extraction buffers were successful in maintaining satisfactory activity for extracts prepared from needle tissues (Table 12).

When analyzed by the gel and electrode buffer "H" (Table 2), five bands of EST activity with R_fs of 0.12, 0.19, 0.33, 0.37, and 0.42 were detected for needles. The band of activity with R_f 0.42 was not detected for preparations from bud tissue, while for bark extracts, the bands of activity with R_fs of 0.19 and 0.42 were not present. Activity was best for needles (Table 12). Results when using gel and electrode buffers "B" and "C" (Table 2) were not as successful.

Activity of F-EST was good for most bud and needle extracts (Table 12). Only one band of activity with R_f 0.65 was observed for buds, while four bands, with R_fs of 0.13, 0.27, 0.57, and 0.65, were detected for extracts prepared from needles (Tables 13,15). Only one weakly staining band with R_f 0.27 was observed for bark.

Only one band of β-GAL activity with an R_f of 0.15 was observed for extracts from bud and bark tissues. Activity was satisfactory for only a few bud extracts (Table 12). No activity was detected for preparations for needles.

Two bands of β-GLU with R_fs of 0.24 and 0.59 were observed for extracts for bud and bark tissues. Only one band of activity

with R_f 0.59 was detected for preparations from needles. Activity was satisfactory for most bud and needle extracts (Table 12).

Activity of GLUDH was poor for all extracts from bud, needle and bark tissues (Table 12). Two bands of activity with R_fs of 0.18 and 0.25 could be detected for extracts from buds and needles, while only one band with R_f 0.25 was observed for preparations from bark tissue.

Four bands of G6PD activity, with R_fs of 0.24, 0.28, 0.33, and 0.37, were observed for preparations from bud and bark tissues. For needles, the bands of activity with R_fs of 0.37 and 0.33 were not detected, while a band with R_f 0.20 was observed. As shown in Table 12, the type of extraction buffer used was important in obtaining satisfactory results.

Only one band of GDH activity with an R_f of 0.32 was observed for extracts from bud, needle, and bark tissues. Activity was good for all bud extracts. However, the type of extraction buffer used was important in maintaining good enzyme activity for extracts from needle and bark tissues (Table 12).

Activity of GPT was best for bud tissue. Two bands of activity were detected. One band, with R_f 0.55, had very high levels of activity, while the second band with R_f 0.15 had poor activity. Only one band of activity with R_f 0.15 was observed for preparations from both needle and bark tissues.

Three bands of G,PDH activity with R_fs of 0.15, 0.21, and 0.25 were observed for extracts from bud tissue. Activity was good for five extracts (Table 12). Only one band, with R_f 0.25, was detected for needle extracts, while two bands of activity with R_fs of 0.21 and 0.25 were detected for preparations from bark tissue. Satisfactory results were obtained for only a few extracts (Table 12).

Most bud extracts contained good activity of α-GPDH (Table 12). One band of activity with R_f 0.27 was detected. Two bands of activity with R_fs of 0.17 and 0.27 were observed for preparations from needle and bark tissues. Satisfactory results were obtained for only a few extracts (Table 12).

Three bands of G_2DH with R_fs of 0.26, 0.30, and 0.34 were detected for all needle extracts (Table 13). Activity was

highest for the middle band. No activity was detected for extracts from bud or bark tissues (Table 12).

Activity of HK was best for extracts from bud tissues (Table 12). Three bands of enzyme activity, with R_fs of 0.25, 0.28, and 0.37, were observed. Two bands of activity with R_fs of 0.25 and 0.37 were detected for preparations for needle and bark tissues. HK activity of the various needle and bark extracts was either poor or not detected (Tables 12-14).

Five bands of IDH activity with R_fs of 0.31, 0.34, 0.37, 0.48, and 0.51 were observed for most bud, needle, and bark extracts (Tables 13-15). The band with R_f 0.34 was the most active. As shown in Table 12, some extraction buffers were more effective than others in maintaining satisfactory enzyme performance.

Activity of LAP was good for most bud extracts. Three bands of activity, with R_fs of 0.61, 0.69, and 0.71, were detected (Tables 12,15). The band of enzyme activity with R_f 0.69 was not detected in extracts prepared from either needle or bark tissues. Some extraction buffers were not effective in maintaining enzyme activity (Table 12).

Five bands of MDH activity with R_fs of 0.23, 0.27, 0.35, 0.39, and 0.44 were observed for extracts from bud tissue. Activity was good for all extracts from bud tissues. The band of activity with R_f 0.44 was not detected for preparations from needle or bark tissues. Only a few extraction buffers were effective in maintaining satisfactory activity for extracts prepared from bark tissue (Table 12).

Activity of ME was best for preparations from bud tissue. Four bands of activity with R_fs of 0.11, 0.16, 0.20, and 0.30 were detected. Two bands of activity with R_fs of 0.20 and 0.34 were detected for needle extracts, while only one band of activity with R_f 0.20 was observed for preparations from bark tissue. One or more bands may actually be MDH.

Six bands of MR with R_fs of 0.15, 0.22, 0.34, 0.43, 0.47, and 0.52 were observed for most bud extracts. Although the enzyme activity was good for all 16 extracts, resolution was only satisfactory (Table 12). Three bands of activity with R_fs of 0.34, 0.43, and 0.47 was detected for needle extracts, while four bands of activity with R_fs of 0.34, 0.43, 0.47, and 0.52 were observed for extracts prepared from bark tissue. Activity was

satisfactory using several of the 16 extraction buffers (Table 12).

NADHDH activity was satisfactory for extracts from bud tissue, but poor for preparations from needle and bark tissues (Table 12). Four bands of enzyme activity with R_fs of 0.32, 0.49, 0.58, and 0.64 were observed for extracts from bud tissue. Only one band of activity with R_f 0.64 was detected in extracts from needles, while two bands of activity with R_fs of 0.32 and 0.64 were present in extracts from bark tissue (Tables 13,14).

Five bands of NADPHDH activity with R_fs of 0.22, 0.28, 0.38, 0.58, and 0.66 were detected for preparations from both bud and bark tissues. For extracts from needles, an additional band of activity with R_f of 0.53 was detected. Activity and resolution were good for all bud extracts, but only for a few needle extracts (Table 12). Results were consistently poor for extracts from bark tissue.

Bud and bark extracts both displayed five bands of PEP activity with R_fs of 0.32, 0.58, 0.63, 0.71, and 0.75. The bands of activity with R_fs of 0.75 and 0.71 were not detected for needle tissue extracts. Resolution was a problem for both needle and bark extracts (Table 12).

Activity of PER was best for bark tissue (Table 12). Eight bands of activity with R_fs of 0.16, 0.20, 0.56, 0.61, 0.66, 0.69, 0.72, and 0.90 were observed. Needle extracts contained seven bands of activity with R_fs of 0.44, 0.47, 0.56, 0.61, 0.66, 0.83, and 0.90, while buds contained four bands of activity with R_fs of 0.61, 0.66, 0.74, and 0.79 (Tables 13,15).

One band of PC activity with an R_f of 0.39 was observed for extracts from bud, needle and bark tissues. Activity was best for extracts from buds (Table 12).

Three bands of 6PGD activity with R_fs of 0.26, 0.34, and 0.41 were detected for extracts made from bud and bark tissues. The band of activity with R_f 0.34 was not detected for extracts from needles. Activity and resolution of 6PGD were good for all bud extracts, but only satisfactory for a few needle and bark extracts (Table 12).

Extracts from bud, needle, and bark tissues all indicated four bands of PGI activity with R_fs of 0.34, 0.40, 0.45, and 0.55 (Tables 13-15). Activity was good for all bud extracts and

several needle extracts (Table 12). Most of the enzyme activity was concentrated in the band of enzyme activity with an R_f 0.40.

Four bands of PGM activity with R_fs of 0.34, 0.48, 0.51, and 0.68 were observed for bud, needle, and bark preparations. The band with R_f 0.68 displayed the greatest activity. The type of extraction buffer used was important in maintaining suitable levels of enzyme activity for needle and bark preparations (Table 12). There may be an overlap of some bands with that of IDH.

Tissue-specific band of activity were observed for isoenzymes of PPO. Bud extracts displayed three bands of activity with R_fs of 0.66, 0.74, and 0.79; needle extracts three bands of activity with R_fs of 0.47, 0.83, and 0.90; and bark extracts two bands of activity with R_fs of 0.20 and 0.56. Activity was highest for extracts from needles (Table 12).

Two bands of SDH activity with R_fs of 0.21 and 0.28 were usually detected for extracts from bud, needle, and bark tissues. Activity of SDH was good for all bud extracts. The band of activity with R_f 0.28 was not detected for many needle and bark extracts (Tables 13,14).

Activity of SUDH was satisfactory for buds, but was poor for needle and bark tissues. Two bands of activity with R_fs of 0.18 and 0.28 were observed for extracts from all three tissues. However, for many needle and bark extracts, one or both bands could not be detected (Tables 13,14).

Bud tissue extracts indicated the best levels of SOD activity (Table 12). Nine bands of activity with R_fs of 0.40, 0.45, 0.51, 0.58, 0.65, 0.73, 0.82, 0.87, and 0.91 were observed. Five bands of activity with R_fs of 0.45, 0.51, 0.58, 0.73, and 0.78 were detected for extracts from needles, while five bands with R_fs of 0.40, 0.51, 0.65, 0.78, and 0.82 were detected for bark preparations. Activity of all 16 extracts for each tissue was very similar.

Black Spruce

When analyzed by the gel and electrode buffer "A", five isoenzyme bands of ACP with R_fs of 0.03, 0.08, 0.16, 0.21, and 0.24 were observed for extracts made from bud, needle, and bark tissues. Activity was satisfactory for most extracts (Table 16). However, resolution was poor for bands near the origin of the gel. Results were also satisfactory when the gel and electrode buffer "H" was used.

Only one band of ACO activity with R_f 0.24 was detected for extracts from bud, needle, and bark tissues (Tables 17-19). Activity was good for all bud extracts, but poor for extracts from needle and bark tissues (Table 16).

Five bands of AK activity with R_fs of 0.13, 0.17, 0.25, 0.33, and 0.37 were detected for all bud extracts, while only one band with R_f 0.25 was present for preparations from needle and bark tissues. Enzyme activity was satisfactory only for extracts from bud tissue.

Two bands of ADH with R_fs of 0.44 and 0.56 were observed for extracts from bud and bark tissues. However, activity was only good for five bark extracts (Table 16). No enzyme activity could be detected in preparations from needle tissue.

Activity of ALD was best for extracts from bud tissue. Three bands of activity with R_fs of 0.08, 0.11, and 0.17 were observed for all bud extracts. The band with R_f 0.08 was not detected for preparations from needle or bark tissues.

Four bands of AAT activity with R_fs of 0.44, 0.56, 0.68, and 0.72 were observed for extracts from bud, needle, and bark tissues. Activity and resolution was best for preparations from bud tissue. Satisfactory results were also obtained for several needle and bark extracts (Table 16).

Activity and resolution of CTO isoenzymes was similar for all extracts for each tissue. Each tissue also displayed unique bands of activity. Eight bands of activity with R_fs of 0.06, 0.14, 0.17, 0.44, 0.48, 0.87, 0.90, and 0.92 were observed for extracts from buds. Seven bands of activity with R_fs of 0.28, 0.44, 0.48, 0.59, 0.73, 0.76, and 0.78 were detected for preparations from needle tissue, while eight bands of activity with R_fs of 0.06, 0.14, 0.44, 0.48, 0.56, 0.59, 0.62, and 0.78 were observed for bark tissue preparations.

Seven bands of DIA activity with R_fs of 0.33, 0.40, 0.56, 0.59, 0.71, 0.75, and 0.78 were observed for bud tissue. For needle preparations, an additional band of activity with R_f 0.67 was observed, while the band of activity with R_f 0.59 was not detected when compared with bud tissue. For extracts from bark, a band of activity with R_f 0.67 was detected while a band with R_f 0.40 was not detected when compared with bud tissue. Activity was best for extracts from buds (Table 16).

When anlyzed by gel and electrode buffer "C" (Table 2), five bands of EST activity with R_fs of 0.07, 0.13, 0.15, 0.20, and 0.24 were observed for extracts from bark tissue. For buds, the band with R_f 0.24 was not detected, while for needle preparations the bands of activity with R_fs of 0.24 and 0.07 were not detected. Only a few extraction buffers were able to maintain satisfactory levels of enzyme activity using needle and bark tissues (Table 16).

Activity of F-EST was good for all bud extracts and several needle extracts. Two bands of activity with R_fs of 0.35 and 0.53 were observed for both tissues. Enzyme activity was lower for extracts from bark tissue. The band of activity with R_f 0.53 was not detected, while another band of activity with R_f 0.78 was observed in these extracts.

One band of β-GAL activity with R_f 0.15 was observed for both bud and needle preparations. Activity was good for all bud extracts, and satisfactory for several needle extracts. Activity was not detected for extracts made from bark tissues.

Activity of β-GLU was good for several preparations from bud and bark tissues, but consistently poor for extracts from needles (Table 16). Two bands of activity with R_fs of 0.60 and 0.88 were detected for buds, while for extracts from needles the band of activity with R_f 0.88 was not detected. An additional band of activity with R_f 0.12 was present in extracts from bark tissue, while the band with R_f 0.88 could not be observed.

Two bands of GLUDH activity with R_fs of 0.17 and 0.26 were observed for preparations from bud, needle, and bark tissues. Activity of this enzyme was best for extracts using buds (Table 16). Enzyme activity of needle and bark extracts was either poor or not detected (Tables 16-18).

The type of extraction buffer used was important to maintain satisfactory activity and resolution of G6PD (Table 16). Two bands of activity with R_fs of 0.23 and 0.30 were observed for

preparations using bud and needle tissues, while an additional band of activity with R_f 0.50 was detected for bark. The extraction buffers 9-11 produced the highest enzyme activity for all three tissues.

Only one band of GDH activity with R_f 0.29 was observed using bud, needle, and bark tissues as sources of protein. Activity was good for all bud extracts, but only satisfactory for a few needle and bark extracts (Table 16).

Activity of GPT isoenzymes was good for all 16 bud extracts. One wide band of activity with R_f 0.57 was detected. For extracts from needles, one band of activity with R_f 0.25 was observed, while for extracts made from bark, no activity was detected for any of the 16 extracts.

Two bands of α-GPDH activity with R_fs of 0.19 and 0.28 were observed for extracts made from bud, needle, and bark tissues. Activity was satisfactory for all bud extracts. However, activity was either poor or not detected for the various needle and bark extracts (Tables 16-18).

Activity of G_2DH was best for extracts prepared from needle tissue. One band of good activity with R_f 0.21 was observed. Enzyme activity was poor for preparations made from bud and bark tissues. Two bands of activity with R_fs of 0.12 and 0.21 were observed.

Three bands of HK activity with R_fs of 0.14, 0.23, and 0.33 were observed for bud tissue preparations. Activity was satisfactory for all extracts. Only one band of activity with R_f 0.23 was detected for needle and bark extracts. Enzyme activity was poor or not detected for the various extracts from both needle and bark extracts (Tables 16-18).

Three bands of IDH activity with R_fs of 0.28, 0.38, and 0.42 were detected for extracts from bud, needle, and bark tissues. The band with R_f 0.28 displayed most of the activity. Activity was good for all 16 extracts of buds. Activity was satisfactory for only several needle and bark extracts as shown in Table 16.

Activity of LAP was good for all bud extracts and several needle extracts. Three bands of activity with R_fs of 0.64, 0.76, and 0.80 were observed. For bark extracts, the band of

activity with R_f 0.76 was not detected. Enzyme activity was satisfactory for only a few bark extracts (Table 16).

Five bands of MDH activity with R_fs of 0.26, 0.32, 0.38, 0.43, and 0.47 were observed for extracts from bud tissue. Activity was good for all extracts. For extracts from needles, the band of activity with R_f 0.47 was not observed. Activity was good for most extracts (Table 16). Activity and resolution were consistently poor for extracts prepared from bark tissue. The band of activity with R_f 0.26 was not detected in preparations from bark tissues.

Activity of ME was not observed for preparations from bud or bark tissues. For needles, four bands of activity with R_fs of 0.09, 0.12, 0.18, and 0.27 were detected. Sufficient enzyme activity was maintained only for some extracts as shown in Table 16. Overlap of some bands with that of MDH was observed.

Three bands of MR activity with R_fs of 0.52, 0.60, and 0.63 were observed for preparations from bud, needle, and bark tissues. Satisfactory results were obtained for all bud extracts. However, resolution for all 16 needle and bark extracts was poor.

Activity and resolution of NADHDH isoenzymes were poor for bud, needle, and bark extracts. Three bands of activity with R_fs of 0.28, 0.57, and 0.61 was observed for buds, while for bark, the bands of activity with R_fs of 0.57 and 0.28 were not detected. Needle tissue extracts indicated two bands of activity with R_fs of 0.28 and 0.45.

Four bands of NADPHDH activity with R_fs of 0.44, 0.48, 0.55, and 0.59 were observed for extracts from bud tissue. Three bands of activity with R_fs of 0.29, 0.44, and 0.59 were detected for needles, while for bark extracts, the band of activity with R_f 0.29 was not detected. The extraction buffers that maintained the best activity and resolution are listed in Table 16.

Four bands of PEP activity with R_fs of 0.27, 0.47, 0.63, and 0.75 were observed for extracts of bud, needle, and bark tissues. Activity was good for all bud extracts. However, satisfactory activity and resolution were obtained for only a few needle and bark extracts (Table 16).

Activity of PER was best for extracts of bark tissue. Six bands of activity with R_fs of 0.44, 0.48, 0.56, 0.59, 0.78, and 0.90 were observed. For preparations from needles, the bands of activity with R_fs of 0.56 and 0.90 were not observed, while a band of activity with an R_f of 0.28 was detected. For extracts of buds, four bands of activity with R_fs of 0.44, 0.48, 0.87, and 0.90 were observed. All extracts for each tissue displayed similar activities (Table 16).

One band of PC activity with R_f 0.37 was observed for preparations from bud, needle, and bark tissues. Activity was good for all bud extracts, and several of the extracts from needle tissue. Satisfactory results were obtained for only a few bark extracts (Table 16).

Activity and resolution of 6PGD isoenzymes were good for all bud extracts. Four bands of activity with R_fs of 0.30, 0.40, 0.43, and 0.47 were observed. The band of activity with R_f 0.40 was not detected for preparations of needle or bark tissues. Satisfactory results were obtained for only a few extracts (Table 16).

Although activity of PGI was good for most bud, needle, and bark extracts, resolution was only satisfactory or poor (Table 16). For extracts from bark, seven bands of activity with R_fs of 0.26, 0.31, 0.38, 0.43, 0.48, 0.57, and 0.61 were observed. The band of activity with R_f 0.61 was not detected for extracts from buds, and for needles the bands of activity with R_fs of 0.26, 0.57, and 0.61 were not detected.

Three bands of PGM activity with R_fs of 0.26, 0.51, and 0.58 were observed for extracts from bud, needle, and bark tissues. Activity was good for all bud extracts and several extracts from needle tissues (Table 16).

Activity and resolution of PPO isoenzymes was similar for all 16 extracts from each of the three different tissues. Seven bands of activity with R_fs of 0.06, 0.14, 0.44, 0.48, 0.87, 0.90, and 0.92 were observed for extracts from bud tissue; seven bands of activity with R_fs of 0.28, 0.44, 0.48, 0.59, 0.73, 0.76, and 0.78 were detected for needles; and eight bands of activity with R_fs of 0.06, 0.14, 0.44, 0.48, 0.56, 0.59, 0.62, and 0.78 were detected in preparations from bark tissue.

Activity of SDH was best for extracts from bud tissue. Three bands of enzyme activity with R_fs of 0.24, 0.27, and 0.30 were observed. The band of activity with R_f 0.30 was not

detected for extracts from needle or bark tissues. Only a few extraction buffers were able to maintain satisfactory enzyme activity (Table 16).

Activity and resolution of SUDH isoenzymes was poor for all bud, needle and bark extracts. Three bands of activity with R_fs of 0.08, 0.15, and 0.22 were observed for extracts from buds. The band of activity with R_f 0.22 was not detected for bark extracts, while for extracts from needles, only one band of activity with R_f 0.08 was detected.

Activity of SOD isoenzymes was good for all bud and needle extracts. Four bands of activity with R_fs of 0.72, 0.75, 0.79, and 0.86 were observed using extracts from buds. For extracts from needles, the band of activity with R_f 0.75 was not detected, while two additional bands with R_fs of 0.37 and 0.68 were observed. For bark extracts, four bands of activity with R_fs of 0.37, 0.68, 0.72, and 0.79 were observed. Activity and resolution of all extracts for each tissue was very similar.

Tamarack
Activity was not observed for any of the extracts for isoenzymes of ACO, ADH, ALP, CAT, β-GAL, G₃PDH, α-GPDH, and SUDH.

When analyzed by the gel and electrode buffer "B", seven bands of ACP activity with R_fs of 0.15, 0.20, 0.23, 0.36, 0.41, 0.53, and 0.64 were observed for extracts from bud and bark tissues (Tables 22,23). For needles, an additional band of activity with an R_f of 0.60 was detected for needle extracts using extraction buffer 12. Activity was good for all bud extracts, and several of the needle extracts (Table 20). Results were not satisfactory when the gel and electrode buffer "A" (Table 2) was used for analysis of ACP isoenzymes.

Good activity of AK was observed for three of the 16 bud extracts (Table 20). Three bands of activity with R_fs of 0.30, 0.36, and 0.46 were detected for all extracts. For extracts from bark tissue, the band of activity with R_f 0.46 was not observed. No enzyme activity could be detected for extracts from needle tissues.

Two bands of ALD activity with R_fs of 0.17 and 0.22 were observed for extracts made from bud and bark tissues. Only a few extraction buffers were able to maintain satisfactory isoenzyme activity (Table 20). No activity was detected for extracts from needle tissue.

Most bands of AAT activity were observed for extracts from bud tissue. Six bands of activity with R_fs of 0.08, 0.16, 0.54, 0.58, 0.64, and 0.68 were detected. Only three bands of activity with R_fs of 0.54, 0.64, and 0.68 were detected in extracts from needle and bark tissues. However, satisfactory activity could be maintained for only several bud and bark extracts (Table 20).

Activity of CTO was good for extracts from bark tissue, but poor for bud and needle preparations. Five bands of activity with R_fs of 0.20, 0.28, 0.44, 0.49, and 0.59 were observed for extracts from buds; three bands of activity with R_fs of 0.44, 0.49, and 0.59 were detected for preparations from needles; and seven bands of activity with R_fs of 0.44, 0.49, 0.56, 0.59, 0.62, 0.94, and 0.98 were detected for bark tissue preparations (Tables 21-23).

Eleven bands of DIA activity with R_fs of 0.23, 0.27, 0.36, 0.48, 0.52, 0.66, 0.71, 0.75, 0.84, 0.88, and 0.95 were observed for extracts from bud tissue. Only two weakly staining bands of

activity with R_fs of 0.71 and 0.75 were observed for extracts from needles, and five bands of activity with R_fs of 0.27, 0.52, 0.71, 0.75, and 0.95 were detected for preparations from bark tissue. Activity of DIA was best for several bud extracts (Table 20).

When analyzed with gel and electrode buffer system "B", six bands of EST activity with R_fs of 0.20, 0.26, 0.29, 0.57, 0.63, and 0.67 were observed for extracts from buds. For needles, two additional bands of activity with R_fs of 0.36 and 0.43 were detected, while the band of activity with R_f 0.20 was not detected. When compared to extracts from buds, two new bands of activity with R_fs of 0.36 and 0.43 were observed using bark extracts, while the band with R_f 0.26 was not observed. Activity of all 16 different extracts for each tissue was very similar.

One band of F-EST activity with R_f of 0.35 was observed for extracts from bud tissues. Activity and resolution was good for all extracts from bud tissues (Table 20). No enzyme activity was detected for extracts from needle or bark tissues.

Activity of β-GLU was good for all bud extracts. Resolution, however, was consistently poor. One band of activity with R_f of 0.70 was observed for extracts from bud, as well as bark tissues. No activity could be detected for extracts prepared from needle tissues.

Only one band of GLUDH activity with R_f of 0.32 was observed for extracts from bud, needle, and bark tissues. Activity was satisfactory for several bud extracts, but was poor or not present for various needle and bark extracts (Tables 20-23).

Two bands of G6PD activity with R_fs of 0.30 and 0.35 was observed for extracts from bud and bark tissues, while only one band of activity with R_f 0.30 was detected for extracts from needles. Satisfactory results were obtained with the extraction buffers as listed in Table 20.

Activity of GDH was good for most bud extracts and several needle extracts. One band of activity with R_f of 0.34 was observed for extracts from all three tissues.

Two bands of GPT activity with R_fs of 0.14 and 0.57 were observed for extracts from bud tissues. Although the activity was good for all extracts, resolution was poor. Only one band of

activity with R_f 0.14 was detected for extracts from bark tissue, while no activity was detected for extracts from needles.

Activity of G_3DH could not be detected for preparations from needle or bark tissues. Two bands of activity, with R_fs of 0.13 and 0.20, were, however, detected for extracts from bud tissue. However, satisfactory activity and resolution was obtained with only two extracts (Table 20).

One band of HK activity with an R_f of 0.32 was detected for extracts from bud and bark tissues. No enzyme activity was observed in extracts made from needles. Good enzyme activity was maintained for only three bud extracts (Table 20).

Activity of IDH was good for all bud extracts and several bark extracts (Table 20). Two bands of activity with R_fs of 0.30 and 0.33 were observed for extracts from buds, while only one band of activity with R_f of 0.30 was detected for extracts from needle and bark tissues.

Two bands of LAP activity with R_fs of 0.67 and 0.80 were observed for extracts from bud and bark tissues. No enzyme activity could be detected for extracts from needles. Activity was optimized for four extraction buffers using buds as a source of protein (Table 20).

Activity of MDH was good for all bud extracts, and several needle and bark extracts as shown in Table 20. However, resolution was poor for all needle and bark extracts. Six bands of activity with R_fs of 0.06, 0.10, 0.16, 0.21, 0.29, and 0.33 were observed for preparations from buds. The bands of activity with R_fs of 0.06 and 0.10 were not observed for extracts from needle and bark tissues.

One band of ME activity with an R_f of 0.16 was observed for preparations from bud and bark tissues. Enzyme activity could not be detected in preparations made from needle tissue. Activity of ME, when detected, was poor. This band of activity may be that of MDH.

Activity of MR was good for all bud extracts. Six bands of activity with R_fs of 0.14, 0.21, 0.29, 0.32, 0.36, and 0.41 was observed for all extracts. Only one band of activity with R_f of 0.29 was present for extracts from needle tissues, while four

bands of activity with R_fs of 0.29, 0.32, 0.36, and 0.41 were detected for bark tissue preparations.

One band of NADHDH activity with an R_f of 0.61 was observed for extracts from buds. Activity was poor for all extracts (Table 20). No enzyme activity could be detected for preparations from needle or bark tissues.

Activity of NADPHDH was good for only several needle extracts (Table 20). Two bands of activity with R_fs of 0.32 and 0.35 were observed. Results were satisfactory for a few bud extracts, and consistently poor for extracts from bark tissue. Three bands of activity with R_fs of 0.26, 0.32, and 0.42 were detected for extracts from buds, and only one band of activity with R_f of 0.32 was observed for extracts from bark tissue.

Three bands of PEP activity with R_fs of 0.28, 0.55, and 0.67 were observed for extracts from bud and bark tissues. For bud extracts, the best activity was associated with the band of R_f 0.55, while for bark extracts, the band of activity with R_f 0.28 was the most intensely stained. The activity and numbers of bands for each tissue source varied with the extraction buffer used (Tables 20,22,23). Only one band of activity with R_f 0.28 was detected for needle preparations.

Activity of PER isoenzymes was best for extracts from bark tissue. Five bands of activity with R_fs of 0.44, 0.49, 0.56, 0.59, and 0.94 were observed. Only three bands of activity with R_fs of 0.20, 0.28, and 0.59 were detected for extracts from buds, while three bands of activity with R_fs of 0.44, 0.49, and 0.94 were observed for extracts from needle tissues. Activity and resolution was similar for all extracts for each tissue.

Activity of PC was observed for only a few bud extracts. Only one band of enzyme activity with R_f 0.31 was observed. In general, activity of this enzyme was consistently poor.

Three bands of 6PGD activity with R_fs of 0.28, 0.32, and 0.34 were observed for extracts of bud and bark tissues. Activity was especially good for all bud extracts and several bark extracts (Table 20). Only one band of activity with R_f 0.28 was detected for extracts from needle tissues.

Activity of PGI was good for all bud extracts. However, resolution was not satisfactory. Five bands of activity with R_fs of 0.28, 0.39, 0.54, 0.60, and 0.64 were observed. The

band with R_f 0.64 was not detected for extracts from bark tissues. Only two bands of activity with R_fs of 0.54 and 0.60 were observed for extracts from needle tissues. Although activity was good, resolution was poor for most bark extracts.

Activity and resolution of PGM was good for all bud extracts. Five bands of activity with R_fs of 0.27, 0.31, 0.33, 0.41, and 0.44 were observed. Enzyme activity was also good for several bark extracts as shown in Table 20. The band of activity with R_f 0.27 was not detected. Only two bands of activity with R_fs of 0.41 and 0.44 were observed for extracts from needle tissues. The cathodal bands overlap those of IDH.

Activity of PPO was best for extracts from bark tissues. Five bands of activity with R_fs of 0.44, 0.49, 0.59, 0.94, and 0.98 were observed. The level of enzyme activity was similar for all extracts. Only three bands of activity with R_fs of 0.20, 0.28, and 0.59 were observed for extracts from bud tissues, while two bands of activity with R_fs of 0.44 and 0.49 were detected for extracts from needles.

Two bands of SDH activity with R_fs of 0.19 and 0.26 were observed for 15 of the bud extracts. However, for extract No. 6 the band of activity with R_f 0.26 was not detected and two new bands of activity with R_fs of 0.23 and 0.35 were observed. This situation results from the faster migration of isoenzymes of extract No. 6. Enzyme activity of needle and bark extracts was consistently poor. For preparations from needles, two bands of activity with R_fs of 0.19 and 0.26 were observed, while for bark extracts the banding patterns were similar to that for extracts from buds.

Three bands of SOD activity with R_fs of 0.50, 0.63, and 0.72 were observed for extracts from bud and bark tissues. Activity was similar for all 16 different extracts for each tissue. Activity was best for preparations from bark tissue. Enzyme activity could not be detected using extracts prepared from needles.

DISCUSSION

The results of this study have demonstrated that the use of protective agents is necessary to maintain satisfactory activity of enzymes extracted from vegetative tissues of the five species examined. However, variations were observed in the effectiveness of each of the 16 different extraction buffers tested in maintaining satisfactory enzyme activity.

Extraction with a buffer that contained no protective agents (extraction buffer 1) was suitable to maintain satisfactory activity for many of the enzymes from white and black spruce and tamarack buds (Table 24). As concentrations of phenols and tannins and other compounds capable of enzyme inhibition are low in this meristematic tissue, protective agents would not be essential for most of the enzymes tested. The entire vegetative buds of jack and Scots pine were used as a source for analysis. This material contains other tissues in addition to the "clean" shoot primordium. Thus, protective agents were required for most of the enzymes examined. Although enzyme activity was poor or undetectable for most enzymes following extraction for needle and bark tissues with this extraction buffer, surprisingly, satisfactory activity was still obtained in many cases for CTO, PER, PPO, SOD, and CAT enzymes.

Extraction of enzymes from bud, needle, and bark tissues with the buffer described by Feret (1971) (extraction buffer 2), which contained several enzyme protective agents, was clearly better in maintaining satisfactory activity for many enzymes, especially from needle and bark tissues, as compared to the extraction buffer described previously (Tables 3-24). For some enzymes, additional isoenzyme bands were observed using this buffer. The addition of seven more protective agents to the buffer of Feret (1971) (extraction buffer 3) increased the range of enzymes that had satisfactory enzyme activity for extractions from needle and bark tissues for all species, except Scots pine (Table 24). Thus, for some enzymes there was the requirement for more protective agents. If seven additional protective agents were added to extraction buffer 3, the resulting buffer (extraction buffer 12) proved very effective for Scots pine, giving the highest number of enzymes that had satisfactory activity (Table 24). Extraction buffer 12 was also more effective than buffer 3 for needles and bark of black spruce, but less effective than buffer 3 for jack pine and tamarack needles and white spruce bark. In the latter case, too many protective agents seemed to decrease the efficiency of the buffer.

The buffer described by Craker et al. (1969) (extraction buffer 4), which was used successfully to extract proteins from apple bark (*Pyrus malus* L.) and foliage of American arborvitae (*Thuja occidentalis* L.), was moderately effective for many enzymes of all species of our study, except for Scots pine (Table 24). The addition of several protective agents to this

buffer and reduction of the pH to 7.1, increased the number of enzymes that were of satisfactory activity for all tissues of the five species examined, except for needles of tamarack (Table 24). This buffer (extraction buffer 5) proved to be the best of the 16 extraction buffers for white spruce. It was used successfully to examine the genetic control of allozyme variants in needles and buds of white spruce (Cheliak and Pitel 1983).

Satisfactory enzyme activity was also obtained using the buffer described by Mitton et al. (1979) (extraction buffer 6) for buds of all species, and also for needle and bark tissues of jack pine and white and black spruce. However, it was only moderately successful for needle and bark tissues of Scots pine and tamarack. If the pH of this buffer (originally at 8.2) was adjusted to pH 6.8, the effectiveness of the resulting buffer (extraction buffer 8) was reduced slightly for many enzymes (Table 24). The reason for lowering the pH was to determine the validity of the suggestion of Loomis (1974) that the best pH for extracting plant enzymes is in the range of pH 6.5 to pH 7.2. In our results (for this buffer at least), the higher pH was more effective.

Cheliak et al. (1983) designed an extraction buffer that was successful in extracting enzymes from buds of aspen (Populus tremuloides Michx.). This buffer (extraction buffer 7) was also effective in extracting enzymes from bud tissues of the five conifers in this study. However, enzyme activity was satisfactory for a smaller range of enzymes extracted from needle and bark tissues as compared with extraction buffers 5 or 6 (Table 24).

Of several enzyme protective agents tested, soluble PVP-40 (2% w/v) gave the highest yield of ferredoxin-$NADP^+$ reductase extracted from eastern hemlock (Tsuga canadensis [L.] Carr.) needles (Riov and Brown 1976). In our study, the extraction buffer that consisted of only soluble PVP-40 (2% w/v) (extraction buffer 14) as a protective agent was effective in maintaining satisfactory activity for many enzymes. However, poor results were obtained for needle tissue of tamarack. It is thought that enzyme activity may be low for these juvenile needles. A simple extraction buffer such as this may thus be suitable to obtain satisfactory results for certain enzymes as indicated in Tables 4, 8, 12, 16, and 20. If insoluble PVPP is used instead of soluble PVP (extraction buffer 16), the effectiveness of this buffer in maintaining satisfactory enzyme activity decreased markedly (Table 24). The addition of Tween-80 and PEG to extraction buffer 14 produced extraction buffer 9 that was more effective than the previous buffer for all tissues of jack pine and tamarack and for needles of white spruce. However, it was less effective for needles and bark tissues of Scots pine. Extraction buffer 11 contained nine more protective agents than extraction buffer 9, and was one of the best of the 16 different extraction

buffers for maintaining satisfactory enzyme activity of needle and bark tissues from Scots pine and black spruce.

The extraction buffer described by Wendel (pers. comm.; extraction buffer 13) was moderately effective in maintaining enzyme activity from needle, bud, and bark tissues of the five conifer species examined. However, if soluble PVP is used instead of insoluble PVPP (extraction buffer 10) the effectiveness of the buffer in maintaining enzyme activity was increased. According to Table 24, this buffer was one of the best in maintaining satisfactory activity for a wide range of enzymes extracted from jack pine, white and black spruce, and tamarack.

Although extraction buffer 15 contained nine protective agents, it performed poorly in maintaining satisfactory enzyme activity from all five species. This may have been because of the lack of a detergent or soluble PVP, and/or owing to the protective agents being of the wrong type, combination, or concentration.

In addition tc differences observed in the effectiveness of the various extraction buffers in maintaining satisfactory enzyme activity among needle, bark, and bud tissues of one species, as well as among the five species, both intra- and inter-specific differences were evident for each enzyme in the activity and numbers of isoenzymes (bands) and their resolution. For some enzymes such as GDH, these differences were small or not present. A maximum of one band of activity of GDH was detected for all three tissues for each of the five species studied. However, for other enzymes, such as PER, major differences in the numbers and and activity of the isoenzymes were observed among needle, bark, and bud tissues of one species as well as when comparing isoenzymes of each tissue among each of the five different species.

CONCLUSIONS

Our results have indicated that vegetative tissues of conifers are satisfactory to use as material to conduct surveys of genetic variation in conifers, or for physiological studies. Bud tissue was the best to use as the problem with phenols and tannins in inhibiting enzyme activity was largely avoided. Needle tissue was satisfactory for many enzymes, provided the proper extraction buffer was used. Although bark tissue was poorest in terms of providing a large range of enzymes to work with, it nevertheless may be suitable for some enzymes such as PER, PGI, ACP, PGM, and others. From our results, we recommend the use of buffers 3, 5, 6, 9, 10, 11, and 12 for jack pine; buffer 12 for Scots pine; buffer 5 for white spruce; buffers 5, 6, 10, 11, and 12 for black spruce; and, buffers 3, 9, and 10 for tamarack. The methods presented here also allow the rapid extraction and analyses of many enzymes from a large number of individuals, which is necessary for population genetics studies.

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Table 1. Enzyme systems analysed, their abbreviations, Enzyme Commission (EC) code designations, and gel buffer(s) upon which they were resolved

Enzyme	Abbrev.	Enzyme Commission Code	Buffer System
Acid phosphatase	ACP	3.1.3.2	A,B,C,H
Aconitase	ACO	4.2.1.3	H
Adenylate kinase	AK	2.7.4.3	H
Alcohol dehydrogenase	ADH	1.1.1.1	B
Aldolase	ALD	4.1.2.13	A,H
Alkaline phosphatase	ALP	3.1.3.1	B,H
Aspartate aminotransferase	AAT	2.6.1.1	B
Catalase	CAT	1.11.1.6	B
Cytochrome oxidase	CTO	1.9.3.1	B
Diaphorase	DIA	1.6.4.3	B
Esterase (colorimetric)	EST	3.1.1.1	B,H
Esterase (fluorescent)	F-EST	3.1.1.1	H
ß-Galactosidase	ß-GAL	3.2.1.23	H
ß-Glucosidase	ß-GLU	3.2.1.21	H
Glucose dehydrogenase	GLUDH	1.1.1.47	B,H
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	H
Glutamate dehydrogenase	GDH	1.4.1.3	B
Glutamate-pyruvate-transaminase	GPT	2.6.1.2	H
Glyceraldehyde-3-phosphate dehydrogenase	G_3PDH	1.2.1.12	H
α-Glycerophosphate dehydrogenase	α-GPDH	1.1.1.8	H
Glycerate-2-dehydrogenase	G_2DH	1.1.1.29	C,H
Hexokinase	HK	2.7.1.1	H
Isocitrate dehydrogenase	IDH	1.1.1.42	H
Leucine-amino peptidase	LAP	3.4.11.1	B
Malate dehydrogenase	MDH	1.1.1.37	H
Malic enzyme	ME	1.1.1.40	H
Mannosephosphate isomerase	MPI	5.3.1.8	H
Menadione reductase	MR	1.6.99.2	H
Nicotinamide adenine dinucleotide dehydrogenase	NADHDH	1.6.99.3	B
Nicotinamide adenine dinucleotide phosphate dehydrogenase	NADPHDH	1.6.99.1	B
Peptidase	PEP	3.4.13.11	B
Peroxidase	PER	1.11.1.7	B
Phosphoenol pyruvate carboxylase	PC	4.1.1.31	B
6-Phosphogluconate dehydrogenase	6PGD	1.1.1.44	H
Phosphoglucose isomerase	PGI	5.3.1.9	B
Phosphoglucomutase	PGM	2.7.5.1	H
Polyphenol oxidase	PPO	1.14.18.1	B
Shikimic acid dehydrogenase	SDH	1.1.1.25	H
Succinate dehydrogenase	SUDH	1.3.99.1	H
Superoxide dismutase	SOD	1.15.1.1	B,H

Table 2. Composition of gel and electrode buffers used for starch gel electrophoresis, and electrophoretic conditions

Name	Buffer		Current
	Gel	Electrode	
A	1:19 dilution of electrode buffer	0.04M Citric acid (anhydrous) pH 6.1 (with N-[3-aminopropyl] - morpholine)	50mA
B	0.03M Tris 0.005M Citric acid (anhydrous) 1% electrode buffer pH 8.5 (10X concentrate solution)	0.06M Lithium hydroxide 0.3M boric acid pH 8.1	50mA
C	1:14 dilution of electrode buffer	0.13M Tris 0.043M Citric acid (anhydrous) pH 7.0	50mA
H	0.05M Histidine-HCℓ 1.40mM EDTA (Bring to pH 7.0 with 1.0M Tris) Dilute stock, 4:1	0.125M Tris (Bring to pH 7.0 with 1M citric acid)	50mA

Table 3. Composition of existing and modified extraction buffers. The modified buffers were those in which existing buffers were changed by the authors, or those in which a totally new combination of ingredients was arranged by the authors

Buffer No.	Composition
1.	0.2M Tris-HCℓ, pH 8.0
2.	As described by Feret (1971) 0.1M Tris-HCℓ, pH 8.0 0.5M Sucrose 6mM Cysteine-HCℓ 6mM Ascorbic acid 1% (v/v) Tween 80
3.	Modified from the buffer of Feret (1971) 0.1M Tris-HCℓ, pH 7.5 0.5M Sucrose 6mM Cysteine-HCℓ 6mM Ascorbic acid 1% (v/v) Tween 80 8% PVP (7:1 of 40M and 360M) 0.4mM NAD 0.1% Bovine Serum Albumin 1mM Dithiothreitol 0.5mM EDTA 1% Tergitol 15-S-9 0.7% β-mercaptoethanol
4.	As described by Craker et al. (1969) 0.05M Borate buffer (pH 9.8) 1% Tergitol 15-S-9 1% PEG (20M) 0.3% β-mercaptoethanol
5.	Modified from the buffer of Craker et al. (1969) 0.05M Borate buffer (pH 7.1) 2% Tergitol 15-S-9 2% PEG (20M) 8% PVP (7:1 of 40M and 360M) 50mM Ascorbic acid 0.4mM NAD 0.1% Bovine Serum Albumin 0.2mM Pyridoxal 5'-phosphate 0.3M Sucrose 12mM Cysteine - HCℓ 0.7% β-mercaptoethanol

6. As described by Mitton et al. (1979)

Dissolve 0.17 g of germanium dioxide in 120 ml boiling distilled water. Cool, and add:
- 0.55g Diethyldithiocarbamic acid, sodium salt
- 8.0g PVP
- 2.2g Sodium tetraborate
- 0.6g Sodium metabisulfite
- 8.0g Ascorbic acid, sodium salt
- 16ml, 0.16M phosphate buffer, pH 7.0

Adjust the volume to 150 ml with distilled water, and stir. Then add
- 16ml Dimethyl sulfoxide
- 1ml 2-phenoxyethanol
- 0.33ml β-mercaptoethanol

Stir and chill in an ice bath

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7. As described by Cheliak (1983)
- 8% PVP (7:1 of 40M and 360M)
- 0.3M Sucrose
- 0.5mM EDTA
- 1mM Dithiothreitol
- 1mM Ascorbic acid
- 0.1% Bovine Serum Albumin
- 0.4mM NAD
- 0.3mM NADP
- 0.2mM Pyridoxal 5'-phosphate

Adjust pH to 6.7 with 1M Tris and add 0.7% β-mercaptoethanol

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8. Buffer is the same as No. 6, except that the pH was adjusted to 6.8

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9. Modified from the buffer of Riov and Brown (1976)
- 0.1M Phosphate buffer (pH 6.8)
- 2% PVP (40M)
- 1% Tween 80
- 2% PEG

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10. Modified from the buffer of Wendel (No. 13)
- 0.1M Phosphate buffer (pH 6.8)
- 5% PVP (4:1 of 40M and 360M)
- 0.15M Ascorbic acid
- 0.02M Diethyldithiocarbamic acid
- 1% β-mercaptoethanol
- 0.02M Sodium metabisulfite
- 0.2M Sodium tetraborate

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11. Modified buffer
0.1M Phosphate buffer (pH 6.8)
2% PVP (40M)
1% Tween 80
2% PEG
0.15M Ascorbic acid
0.02M Diethyldithiocarbamic acid
1% β-mercaptoethanol
0.02M Sodium metabisulfite
0.02M Sodium tetraborate
6mM Cysteine-HCℓ
0.1% Bovine Serum Albumin
0.4mM NAD
1mM Dithiothreitol

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12. Modified buffer
0.1M Tris-HCℓ (pH 7.2)
0.5M Sucrose
6mM Cysteine-HCℓ
6mM Ascorbic acid
1% (V/V) Tween 80
4% PVP (3:1 of 40M and 360M)
0.02M Diethyldithiocarbamic acid
0.06M Sodium tetraborate
30mM Sodium metabisulfite
0.4mM NAD
0.1% Bovine Serum Albumin
1mM Dithiothreitol
0.5mM EDTA
0.2mM Pyridoxal 5'-phosphate
10% Dimethyl sulfoxide
1% 2-phenoxyethanol
1% Tergitol 15-S-9
1% PEG (20M)
1% β-mercaptoethanol

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13. As described by Wendel (personal communication)
0.1M Phosphate buffer (pH 6.8)
0.15M Ascorbic acid
0.02M Diethyldithiocarbamic acid
1% β-mercaptoethanol
0.02M Sodium metabisulfite
0.2M Sodium tetraborate
After homogenization, enough prewashed and dried polyvinylpolypyrrolidone (PVPP) is then added to the homogenate to make a moist paste.

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14. As described by Riov and Brown (1976)
0.1M Phosphate buffer (pH 6.8)
2% PVP (40M)

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15. Modified buffer
0.1M Phosphate buffer (pH 6.8)
30mM Sodium metabisulfite
0.06M Sodium tetraborate
1mM Ascorbic acid
0.02M Diethyldithiocarbamic acid
12mM Cysteine-HCl
0.4mM NAD
0.3mM NADP
1mM Dithiothreitol
0.2mM Pyridoxal 5'-phosphate

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16. Modified buffer
0.1M Phosphate buffer (pH 6.8) with PVPP (insoluble) added to make a moist paste

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Abbrevitions: Tris, Tris (hydroxymethyl) aminomethane; EDTA, ethylenediaminetetraacetate; PEG, polyethylene glycol; PVP, polyvinylpyrrolidone; PVPP, polyvinylpolypyrrolidone; NAD, β-nicotinamide adenine dinucleotide; NADP, β-nicotinamide adenine dinucleotide phosphate.

Table 4. Summary for jack pine of the best extraction buffers to use for each tissue and enzyme, and the type of activity and resolution observed (Act.=activity; Resol.=resolution; P=poor; S=satisfactory; G=good; NA=no activity)

ENZYME	NEEDLES			BARK			BUDS		
	Extraction Buffer No.	Act.	Resol.	Extraction Buffer No.	Act.	Resol.	Extraction Buffer No.	Act.	Resol.
ACP	3-6,8,9,11-14	G	S	1-16	G	S	1-6,8,9,11-14	G	S
ACO	3-6	P	P	5-7,12,14	S	S	2-6,8,11,13,14,16	G	G
AK	3,9,10	P	P	2,3,11-13	P	P	2,3,9,10	S	S
ADH	3,12	S	S	1-3,10,12	S	S	2-5,9-12,14	S	S
ALD	3-6,8-10,12	S	S	5,6,10-12	S	S	1-16	G	S
ALP	8-12	S	S	1-16	G	S	3-5,7-13,15,16	S	S
AAT	3-6,12	S	S	2,3,5,7,10,12,14	S	S	5-7,9-15	G	S
CAT	1-16	P	P	1-16	S	S	1-16	S	S
CTO	2-15	G	S	1-16	S	S	1-16	P	S
DIA	2-6,10-12	P	S	2-6	S	S	2,4,5,7-9,11,14	S	S
EST	2-15	G	S	1-16	S	S	2-15	P	P
F-EST	2-5,9-14	G	S	2-6,9-14	G	S	2,3,5-12	G	G
β-GAL	NA			NA			2-6	G	S
β-GLU	9-14	P	P	1-16	P	P	1-16	S	S
GLUDH	3,12	S	S	2,3,10,12	S	S	2,3,12	S	S
G6PD	9-11	S	S	10,11	S	P	4,5,9,11	S	S
GDH	3,6,8,10-12	G	G	4-6,8,9	S	S	2-15	G	G
GPT	2-5,12-14	P	P	4-6,9-14	P	P	2-5,9-14	G	P
G_3 PDH	3-6,8	P	P	5,9,11	P	P	3-6,10-12	S	S
α-GPDH	2-6,8,10-14	P	P	2-6,8-14	P	P	1-16	P	S
G_2 DH	4-6,8-12	G	G	NA			NA		
HK	6,7	P	P	5-7	P	P	1-16	S	G
IDH	3,6,8-10	S	S	6,8-14	S	S	2-15	G	G
LAP	2,3,6,9,10	S	S	5,9-11,14	S	S	2,5,9,11	G	G
MDH	3-6,9-11,14	S	S	5,6,8,9,11-13	S	P	2,5,6,9,11,13,16	G	G
ME	3,4,8-11	S	S	3,4,9-11	S	S	3-5,7-11	S	S
MPI	NA			2-5	P	P	2-6,8,10	S	S
MR	2-4,6,8-12	P	P	2,4,6,7	S	P	2,4,6,7,9-11,14-16	G	S
NADHDH	3,5,6,9,10	P	P	NA			2-6,8-14	S	S
NADPHDH	3,5,6,8-12	S	S	9-13	P	P	5-13	G	G
PEP	2-6,8-13	P	P	2-5,8-15	S	S	1-16	S	S
PER	2,3,6,8-13,15	G	G	1,2,6-10,12	G	S	1-4,8-10,14,16	S	S
PC	5,6,10-14	P	S	10-14	P	P	3-6,9,11-14	S	S
6PGD	3,6,10	S	S	5,9,14	S	S	2,4-9,11,14	G	G
PGI	2-6,8,12-14	G	S	3-5,9	G	P	5-9,11-15	G	S
PGM	3-6,8-14	G	P	4-6,9-14	G	S	4-7,9,11,14	G	S
PPO	1-16	S	S	1,2,5-16	S	S	1-16	S	S
SDH	2-6,8-10,14	S	S	5,7-12,14	S	P	5,6,9,11,14	G	S
SUDH	3	S	S	3,5,6,9-16	P	P	3-5,9-16	S	S
SOD	1-16	G	S	1-16	S	S	1-16	G	S

Table 5. Number of isoenzyme bands observed for extracts prepared from jack pine needles using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5
ACO	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0
AK	0	2	3	0	0	0	0	1	3	3	2	2	2	0	0	0
ADH	0	1	2	1	1	0	0	0	0	0	1	1	0	0	0	0
ALD	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ALP	3	3	4	4	4	4	4	5	5	5	4	5	4	5	5	5
AAT	1	3	4	3	3	3	3	3	3	2	2	3	3	3	2	2
CAT	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CTO	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
DIA	2	5	6	5	5	4	4	4	2	6	6	6	3	5	2	2
EST	0	3	4	4	3	3	3	3	4	4	4	3	3	3	3	3
F-EST	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
β-GAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-GLU	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GLUDH	0	3	3	1	1	0	0	0	0	0	3	3	0	0	0	0
G6PD	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
GDH	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G_3PDH	0	1	2	2	1	1	1	1	1	1	0	0	0	0	0	0
α-GPDH	0	2	2	2	2	2	0	2	0	2	2	2	0	2	0	0
G_2DH	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HK	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
IDH	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LAP	1	2	2	2	2	2	1	2	2	1	1	1	1	1	1	1
MDH	2	3	3	3	2	2	2	2	3	3	3	3	3	3	2	1
ME	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
MPI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MR	0	4	4	4	2	4	3	3	3	3	3	3	0	3	0	0
NADHDH	0	3	3	3	3	3	1	1	3	3	2	1	1	1	1	0
NADPHDH	0	0	2	2	2	2	2	2	2	2	2	2	2	2	1	1
PEP	3	4	4	4	4	4	2	3	3	3	3	3	3	3	2	2
PER	8	9	9	9	9	9	8	9	9	9	9	9	9	9	9	9
PC	0	0	0	0	1	1	0	0	0	1	1	1	1	1	0	0
6PGD	0	2	2	2	2	2	0	0	2	2	2	2	2	2	0	0
PGI	0	2	3	2	2	2	2	2	2	2	2	2	3	3	3	3
PGM	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0
PPO	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
SDH	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0
SUDH	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD	3	4	4	4	4	4	4	4	4	4	4	5	5	5	4	3

Table 6. Number of isoenzyme bands observed for extracts prepared from jack pine bark using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	6	6	6	6	6	5	6	6	6	6	6	6	6	6	6	6
ACO	0	0	2	2	2	2	2	2	2	1	1	2	1	2	0	0
AK	0	2	2	1	0	0	0	0	1	1	2	2	2	0	0	0
ADH	2	2	2	1	2	1	1	1	1	2	1	2	1	1	1	1
ALD	0	1	1	1	2	2	1	1	1	3	3	3	1	1	1	1
ALP	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
AAT	3	4	4	2	4	4	4	3	3	4	3	4	3	3	3	3
CAT	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CTO	9	9	9	7	7	9	7	8	8	9	7	7	7	9	9	9
DIA	5	5	5	5	5	5	4	4	4	4	5	5	4	5	4	4
EST	3	3	4	4	3	3	3	3	3	4	4	4	3	4	3	4
F-EST	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
β-GAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-GLU	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
GLUDH	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
G6PD	0	1	2	2	2	2	1	1	1	2	2	2	2	2	1	0
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G_3 PDH	0	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0
α-GPDH	0	2	2	2	2	2	0	2	2	2	2	2	0	2	0	0
G_2 DH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HK	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
IDH	0	1	1	1	1	2	1	2	2	2	2	2	1	1	1	1
LAP	2	2	2	2	3	2	2	3	3	3	3	3	3	3	3	2
MDH	3	3	3	3	3	3	3	3	3	3	3	4	4	4	3	3
ME	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
MPI	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
MR	2	4	4	4	4	4	4	4	4	4	4	4	4	3	4	3
NADHDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NADPHDH	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0
PEP	0	6	6	6	6	6	4	5	5	6	6	6	5	5	5	0
PER	9	9	9	8	8	9	8	8	9	9	9	8	7	8	8	8
PC	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0
6PGD	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0
PGI	4	4	5	5	5	4	4	4	5	4	4	4	4	4	4	4
PGM	2	3	3	3	3	3	2	2	3	3	3	3	3	3	3	2
PPO	3	3	2	2	3	3	3	3	3	3	3	3	3	3	3	3
SDH	0	2	2	2	1	3	3	2	2	2	2	2	2	2	2	0
SUDH	0	0	1	0	1	1	0	0	1	1	1	1	1	1	1	1
SOD	4	4	4	4	3	4	4	4	3	3	3	5	5	5	4	3

Table 7. Number of isoenzyme bands observed for extracts prepared from jack pine buds using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	5	5	3	6	6	6	6	6	6	5	5	5	5	5	5	5
ACO	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
AK	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
ADH	1	2	2	2	1	2	2	2	1	1	1	2	2	1	2	2
ALD	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3	3
ALP	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
AAT	4	4	4	4	4	5	4	4	4	4	4	4	4	4	4	4
CAT	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
CTO	7	7	7	6	5	6	6	7	6	7	6	5	5	6	6	6
DIA	8	9	9	9	9	9	7	7	7	6	9	8	8	9	7	7
EST	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0
F-EST	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
β-GAL	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
β-GLU	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
GLUDH	1	3	3	2	1	1	1	1	0	1	2	3	0	0	0	0
G6PD	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G_3 PDH	2	2	3	3	3	3	2	3	3	3	3	3	3	3	2	2
α-GPDH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G_2 DH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HK	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
IDH	1	2	2	1	2	1	2	2	2	2	1	1	1	1	1	1
LAP	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2
MDH	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
ME	2	3	3	4	4	3	4	4	4	4	4	3	3	3	3	2
MPI	2	3	3	3	3	3	3	3	3	3	3	2	2	3	3	2
MR	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5
NADHDH	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
NADPHDH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PEP	5	5	6	6	6	5	5	5	5	5	5	5	5	5	5	5
PER	7	7	7	6	5	6	6	7	7	7	6	6	6	6	6	6
PC	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6PGD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PGI	2	2	2	2	3	2	3	3	3	3	3	3	3	3	3	3
PGM	3	3	3	5	3	3	3	3	3	3	3	3	5	5	5	3
PPO	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
SDH	3	3	3	3	2	3	2	3	2	3	3	3	2	3	3	3
SUDH	1	1	2	2	2	1	1	1	2	2	2	2	2	2	2	2
SOD	3	3	3	3	3	3	3	3	4	4	4	3	4	3	3	3

Table 8. Summary for Scots pine of the best extraction buffers to use for each tissue and enzyme, and the type of activity and resolution observed (Act.=activity; Resol.=resolution; P=poor; S=satisfactory; G=good; NA=no activity)

ENZYME	NEEDLES			BARK			BUDS		
	Extraction Buffer No.	Act.	Resol.	Extraction Buffer No.	Act.	Resol.	Extraction Buffer No.	Act.	Resol.
ACP	2,3,8-14,16	S	P	2-6,10-14	S	P	9,11,12,14,15	G	S
AK	11,12	S	S	11,12	P	P	4-14	G	G
ADH	3,12	P	P	3,11,12	P	P	4-7,9-15	G	S
ALD	4-8,10-12	G	S	5,6,10-12	S	S	3,4,6,10,12-14	G	S
AAT	2,3,5,7,10,12,14	S	S	3,5-7,10,12,14	G	S	4-7,9,10	G	G
CAT	1-16	S	S	1-16	P	S	1-5	P	P
CTO	1,2,6-16	S	S	1,2,6-16	S	S	1-16	S	S
DIA	3,5,7,8,10	P	S	5,6,8,12	P	S	2,3,5,7,10-14	G	S
F-EST	9-14	G	S	2,3,9-14	S	S	6,9-15	G	G
β-GAL	NA			NA			1-16	G	S
β-GLU	2-15	P	P	2-15	P	P	2,3,5,6,8,9,12,14	G	S
GLUDH	NA			12	S	S	2-6,10-15	S	S
G6PD	11	G	S	4,5,7,9-11	S	S	8,10,11,13	G	G
GDH	2,3,5-8,10,12,13	G	G	5,6,10,13	S	G	2,3,5-14	G	G
GPT	11-14	S	P	NA			3,10-14	G	P
α-GPDH	12-14	S	S	12	S	S	6,8-13	S	S
G_2DH	2,5,6,12-15	S	S	12	P	S	5,7,9-14	P	P
HK	5,6,11-14	P	P	5,11-14	P	P	2-15	S	S
IDH	5,6,11-15	G	G	5,6,8-14	G	G	5-15	G	G
LAP	11-15	G	S	3-7,9-15	S	S	1-16	G	G
MDH	2,3,5,11-15	G	S	5,8,9,11-14	S	S	2,5-14	G	G
ME	10-14	S	P	12	P	S	5-14	G	G
MR	2-14	P	P	2-14	P	P	8,9,11	G	G
NADHDH	NA			NA			3-14	G	G
NADPHDH	10-13	S	S	NA			1-16	P	P
PEP	5,9,10,12-14	S	S	9,11,13,14	P	S	9,11-13	S	S
PER	1-3,5-16	G	G	1,2,6-16	S	S	1-3,5-16,	G	G
PC	11-14	S	G	10-14	P	S	5-15	G	G
6PGD	5,11-15	S	S	5,10-12,14	S	S	8-15	G	G
PGI	2,3,5,11,12,14	G	S	2,3,5,11,12,14	S	P	5,10-13	G	S
PGM	4-6,9-11,14	G	P	4,5,11,14	G	P	5,7,9-14	G	S
PPO	1-16	G	S	1,2,6-16	S	S	1-16	G	S
SDH	2,5,6,8-15	G	G	3,5,8-14	S	S	2,4-15	G	G
SUDH	12	S	P	12	S	S	2,3,5,9,11-13,15	G	S
SOD	1-16	S	S	1-16	S	S	1-16	G	S

Table 9. Number of isoenzyme bands observed for extracts prepared from Scots pine needles using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	6	9	9	9	9	9	9	9	9	10	10	10	10	10	10	10
AK	0	0	1	1	1	1	0	0	0	1	2	2	1	1	1	0
ADH	1	1	2	2	2	0	2	1	0	1	1	2	1	1	1	0
ALD	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AAT	3	9	9	5	9	4	8	3	3	6	4	9	5	9	6	6
CAT	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
CTO	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
DIA	0	2	3	3	3	2	3	3	2	3	2	2	3	3	2	1
F-EST	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
β-GAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-GLU	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
GLUDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G6PD	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0
α-GPDH	0	0	0	0	0	0	0	0	0	0	4	4	4	2	2	2
G_2DH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HK	0	0	0	0	1	1	0	0	0	0	1	1	1	1	0	0
IDH	0	0	0	1	3	3	1	1	1	1	3	3	3	3	1	1
LAP	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
MDH	1	5	5	5	4	4	4	4	5	6	7	7	6	7	6	3
ME	0	3	3	3	3	3	2	3	3	3	3	3	3	3	3	0
MR	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
NADHDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NADPHDH	0	2	2	2	2	2	2	2	2	2	2	2	2	2	0	1
PEP	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	3
PER	5	5	5	5	5	5	5	5	5	5	5	5	6	6	5	5
PC	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0
6PGD	1	1	1	1	2	1	1	1	1	1	2	2	2	2	2	1
PGI	2	4	4	3	4	3	3	3	3	3	4	4	3	3	3	2
PGM	0	0	3	3	3	3	2	3	3	3	3	3	3	3	3	3
PPO	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
SDH	0	2	1	1	2	2	1	2	2	2	2	2	2	2	2	1
SUDH	0	3	3	0	0	0	0	0	0	0	0	5	5	5	3	0
SOD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Table 10. Number of isoenzyme bands observed for extracts prepared from Scots pine bark using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	6	11	11	11	11	6	5	6	6	8	8	8	8	11	6	5
AK	0	0	1	1	1	1	0	1	1	1	1	1	1	1	0	0
ADH	0	0	2	2	2	1	1	1	1	1	1	2	1	1	1	0
ALD	1	2	1	1	2	2	1	1	1	2	2	2	2	2	2	1
AAT	3	6	9	4	9	8	9	6	4	9	6	9	5	7	5	5
CAT	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
CTO	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
DIA	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0
F-EST	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
ß-GAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ß-GLU	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
GLUDH	0	0	1	1	1	1	1	1	1	2	2	2	1	1	1	0
G6PD	0	0	1	1	2	1	2	2	2	2	2	1	1	1	1	1
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
GPT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-GPDH	2	2	2	2	2	0	0	0	0	2	2	4	4	2	0	0
G_2DH	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
HK	0	0	0	0	1	0	0	0	0	0	1	1	1	1	0	0
IDH	0	1	1	1	2	2	2	2	2	2	2	2	2	2	1	1
LAP	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
MDH	0	3	4	4	3	3	3	4	4	3	4	4	3	4	3	1
ME	0	2	2	2	3	3	3	3	3	3	3	3	3	3	0	0
MR	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
NADHDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NADPHDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PEP	0	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0
PER	5	5	4	3	3	5	3	4	4	4	4	4	4	4	4	4
PC	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0
6PGD	0	1	1	1	2	1	1	1	1	2	2	2	2	2	1	1
PGI	4	5	5	5	5	5	5	5	5	5	5	5	4	4	3	2
PGM	2	0	0	3	3	3	3	3	3	3	3	3	3	3	3	2
PPO	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
SDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SUDH	0	5	5	5	5	5	0	0	0	5	5	5	5	5	0	0
SOD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Table 11. Number of isoenzyme bands observed for extracts prepared from Scots pine buds using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	6	6	6	6	6	6	6	8	8	8	8	8	6	6	6	4
AK	1	1	1	1	1	1	1	1	1	2	2	2	1	1	1	1
ADH	1	2	2	2	2	2	2	1	2	3	3	2	2	2	2	1
ALD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
AAT	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
CAT	3	3	3	3	3	0	0	0	0	0	0	0	0	0	0	0
CTO	10	10	8	8	9	10	10	10	10	10	10	10	10	10	10	10
DIA	5	6	8	8	8	8	8	8	6	8	8	8	8	8	6	6
F-EST	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
β-GAL	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
β-GLU	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GLUDH	0	2	2	2	2	2	0	1	1	2	2	2	2	2	2	0
G6PD	1	1	1	1	1	1	1	1	1	3	3	2	3	1	1	1
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
α-GPDH	2	2	2	2	2	4	4	4	4	4	4	4	4	4	2	2
G_2DH	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
HK	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
IDH	2	2	2	2	4	2	2	4	4	4	4	4	4	4	4	1
LAP	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
MDH	2	6	6	6	6	5	6	6	6	7	7	6	7	7	7	1
ME	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
MR	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1
NADHDH	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
NADPHDH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PEP	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PER	9	9	9	7	9	9	9	9	9	9	9	9	9	9	9	9
PC	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
6PGD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PGI	5	5	5	5	5	4	5	5	5	5	5	5	5	5	4	2
PGM	2	3	3	3	5	3	5	3	5	4	4	4	4	4	4	3
PPO	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
SDH	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1
SUDH	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SOD	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Table 12. Summary for white spruce of the best extraction buffers to use for each tissue and enzyme, and the type of activity and resolution observed (Act.=activity; Resol.=resolution; P=poor; S=satisfactory; G=good; NA=no activity)

ENZYME	NEEDLES			BARK			BUDS		
	Extraction Buffer No.	Act.	Resol.	Extraction Buffer No.	Act.	Resol.	Extraction Buffer No.	Act.	Resol.
ACP	5,10,11,13,14	S	S	9,11,12,13-15	S	S	1-16	G	S
ACO	5,10,14	S	S	5,6,14	P	P	1,2,4-11,14,15	G	S
AK	9-13	P	P	2-5,10-12	P	P	4-15	S	S
ADH	9-11	P	P	3-6,10-15	P	P	4,5	S	G
ALD	4-6,9-13	S	S	4-6,10-13	S	S	1-16	S	S
ALP	2-15	P	P	3-5,11,12	P	P	1-16	S	S
AAT	2-6,11,12	G	S	2,3,5,7,10,12, 14,15	G	S	1-16	G	G
CAT	NA			2-5,9-11	S	S	1-16	S	S
CTO	1-3,6,8,9,12-16	G	S	1-16	G	S	1-16	P	S
DIA	5,6,12	S	S	2-5,8-11,14,15	S	S	1-16	G	S
EST	2-15	S	S	2-6,11-14	P	P	3-6,10-14	P	P
F-EST	2-13	G	S	2-15	P	P	1-16	G	G
β-GAL	NA			2-6,11-14	P	P	2-6	G	S
β-GLU	9-14	S	P	2-6,8-14	P	P	1-16	S	P
GLUDH	2-15	P	P	3-14	P	P	3-14	P	P
G6PD	4,5,9-12	S	S	4,10-15	S	S	4,5,8,9	G	S
GDH	2,5,6,8,10,12	G	G	6,8,10-13	G	G	1-16	G	G
GPT	2-7,9-14	S	S	9-14	P	P	1-6	G	P
G_3 PDH	4,5,7,8,14	S	G	3,5,8	P	S	5,6,9,11,14	G	G
α-GPDH	2-6,10-14	S	S	2-5,11-15	S	S	1-6,9-14	G	P
G_2 DH	3-6,8-10,12,13	S	S	NA			NA		
HK	5,9	P	P	3-6,11-14	P	P	1-16	G	G
IDH	4-6,9-11	G	S	3-15	S	P	3-8,10,14,15	G	G
LAP	1,2,4-6,9-14	S	S	9-11,13,14	S	S	4-6,9,11,14,15	G	G
MDH	3-14	G	G	3,5,7,9,10,13	S	S	1-16	G	G
ME	3,4,9-13	P	P	4,5,8-11	P	P	3-5,8,9,11-14	S	G
MR	2-5,9,10	S	P	2-6,8,10,14	S	P	1-16	G	S
NADHDH	2-6,8-14	P	P	5,6,9,10	P	P	2-7,10,11,15	S	S
NADPHDH	3,5,6,8,9	G	G	3-6,9-14	P	P	1-16	G	G
PEP	3-5,9-11	S	P	3-5,9-11	S	P	1-16	S	S
PER	1-3,6,8,9,15,16	S	S	1-3,5,6,8,9,14 16	G	S	1-16	P	S
PC	5,9-11	P	S	5,6	S	S	1-16	G	G
6PGD	4,5,9,10	S	S	3-5,7,9-11	S	S	1-16	G	G
PGI	2,3,5,6,8,9, 12,14	G	S	3-7,10-12	S	P	1-16	G	S
PGM	4,5,9-11,13,14	G	S	4,5,7,9-11	G	S	1-16	G	S
PPO	1-3,6,15,16	S	S	1-16	P	P	1-16	P	S
SDH	3,5,8-10,12	P	P	3,5-10,13	S	S	1-16	G	S
SUDH	2,3,10-14	P	P	2,3,11,12	P	P	3-5,8,10,13	S	S
SOD	1-14	S	S	1-10	S	S	1-16	G	G

Table 13. Number of isoenzyme bands observed for extracts prepared from white spruce needles using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	0	3	6	6	6	6	6	6	6	6	6	6	6	6	6	0
ACO	0	2	2	2	2	2	2	2	2	2	2	2	2	2	0	0
AK	0	2	2	2	2	0	0	0	3	3	3	3	3	2	0	0
ADH	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
ALD	1	1	2	3	3	3	2	1	2	2	2	2	2	1	1	1
ALP	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AAT	0	4	4	2	4	2	2	2	3	2	4	4	2	2	2	2
CAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CTO	7	7	7	5	5	7	5	7	7	5	5	7	7	7	7	7
DIA	1	6	7	6	8	8	7	6	6	6	7	8	6	6	5	3
EST	4	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4
F-EST	3	4	4	4	4	4	4	4	4	4	4	4	4	3	3	3
ß-GAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ß-GLU	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GLUDH	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0
G6PD	0	1	2	2	3	1	1	1	2	2	2	1	1	1	1	1
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
G_3PDH	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	0
α-GPDH	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G_2DH	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
HK	0	2	2	2	2	1	1	2	2	2	2	2	1	1	0	0
IDH	0	5	5	5	5	5	2	4	5	5	5	5	5	5	5	3
LAP	2	2	2	2	2	2	2	1	2	2	2	2	2	2	1	1
MDH	3	3	4	4	4	4	4	4	4	4	4	4	4	4	3	3
ME	0	1	1	1	1	1	1	1	2	2	2	2	2	1	1	0
MR	2	3	3	3	3	3	3	3	3	3	2	3	3	2	2	2
NADHDH	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0
NADPHDH	0	4	6	6	6	6	6	6	6	6	6	6	5	4	5	4
PEP	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
PER	7	7	7	6	6	7	5	7	7	7	7	7	7	7	7	7
PC	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0
6PGD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PGI	2	3	4	4	4	4	3	4	4	3	4	4	4	4	4	4
PGM	0	4	4	4	4	4	3	3	4	3	3	3	3	4	3	1
PPO	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SDH	0	1	2	1	1	2	1	1	2	2	2	2	1	1	0	0
SUDH	0	2	2	0	0	0	0	0	0	2	2	2	2	2	0	0
SOD	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 14. Number of isoenzyme bands observed for extracts prepared from white spruce bark using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	2	5	5	6	6	3	4	3	6	5	6	6	6	6	6	5
ACO	0	0	2	2	2	2	2	2	2	2	2	0	0	2	2	0
AK	0	3	3	2	2	0	1	0	0	3	3	2	2	2	0	0
ADH	0	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0
ALD	1	1	1	2	2	2	1	2	1	3	3	3	2	1	1	1
ALP	0	1	2	2	2	2	1	1	1	1	2	2	1	2	1	0
AAT	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
CAT	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CTO	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
DIA	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
EST	0	3	3	3	3	3	0	0	0	2	3	3	3	3	0	0
F-EST	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
β-GAL	0	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0
β-GLU	1	2	2	2	2	2	1	2	2	2	2	2	2	2	1	1
GLUDH	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0
G6PD	0	1	2	4	3	3	2	2	3	4	4	4	4	3	3	2
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0
G_3 PDH	0	2	2	2	2	1	0	2	2	2	2	0	2	0	0	0
α-GPDH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G_2 DH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HK	0	0	1	1	1	1	0	1	1	1	2	2	2	2	0	0
IDH	0	5	5	5	5	5	5	5	5	5	5	3	5	5	5	1
LAP	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1
MDH	3	3	3	3	3	3	3	3	3	3	3	3	4	3	3	3
ME	0	0	1	1	1	1	0	1	1	1	1	0	0	0	0	0
MR	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3
NADHDH	0	1	1	1	1	1	1	1	1	1	1	1	2	1	1	0
NADPHDH	0	2	5	4	5	4	2	2	5	5	5	4	5	5	2	1
PEP	5	5	5	5	5	4	4	4	5	5	5	4	4	4	4	2
PER	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
PC	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6PGD	2	3	3	3	3	2	3	3	3	3	3	3	3	3	2	2
PGI	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	3
PGM	1	1	3	4	4	4	4	4	4	4	4	4	4	4	2	2
PPO	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
SDH	0	1	1	1	1	2	1	1	1	2	1	1	1	1	1	1
SUDH	0	2	2	1	1	1	1	1	1	2	2	2	2	2	1	0
SOD	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4

Table 15. Number of isoenzyme bands observed for extracts prepared from white spruce buds using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	4	4	6	5	6	5	6	6	6	6	6	6	6	6	6	6
ACO	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
AK	5	6	6	6	5	3	3	3	3	3	3	3	4	4	4	3
ADH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ALD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
ALP	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
AAT	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
CAT	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
CTO	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
DIA	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
EST	0	0	4	4	4	4	0	0	1	4	4	4	4	4	0	0
F-EST	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
β-GAL	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
β-GLU	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
GLUDH	1	1	2	2	2	2	2	2	2	2	2	2	2	2	1	1
G6PD	4	4	4	4	3	3	3	3	3	4	4	3	4	4	4	3
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G_3 PDH	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	2
α-GPDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G_2 DH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HK	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2
IDH	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
LAP	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3
MDH	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
ME	3	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3
MR	6	6	5	6	6	6	6	6	6	6	6	6	6	6	6	5
NADHDH	3	4	4	4	4	4	4	3	3	4	4	3	3	3	4	3
NADPHDH	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PEP	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PER	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PC	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6PGD	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
PGI	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PGM	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PPO	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SDH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
SUDH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
SOD	10	10	10	9	10	8	7	8	8	8	9	9	9	8	7	8

Table 16. Summary for black spruce of the best extraction buffers to use for each tissue and enzyme, and the type of activity and resolution observed (Act.=activity; Resol.=resolution; P=poor; S=satisfactory; G=good; NA=no activity)

ENZYME	NEEDLES		BARK		BUDS	
	Extraction Buffer No.	Act. Resol.	Extraction Buffer No.	Act. Resol.	Extraction Buffer No.	Act. Resol.
ACP	4-14	S P	4,6,8-14	S P	1-16	S S
ACO	5,6	P S	6	P S	1-16	G G
AK	5,6,9-12	P S	3,4,9-11	P P	1-16	S S
ADH	NA		11-15	G S	1-16	S S
ALD	4-6,8-14	G S	4-6,10-12	G S	1-16	G S
AAT	2-7,10,12,14	G S	3-6,10,12,14,15	S S	1-16	G G
CTO	1-16	S S	1-16	S S	1-6	S S
DIA	3,4,8,10-12	S S	10-12	S S	1-16	G S
EST	11,12	S P	9-12	S P	1-16	S S
F-EST	4-6,9-15	G S	1-16	S S	1-16	G G
β-GAL	3-6,8-14	S S	NA		1-16	G S
β-GLU	3-6,8-12,14	P S	2,3,5,10-14	G P	2-6,11,12	G S
GLUDH	3-6,11-14	P P	3,10-14	P P	3-14	S S
G6PD	9-11	S S	9-11	S P	5,9-11,13-15	S S
GDH	3,6,8,10-14	S S	10,12	S S	1-16	G G
GPT	5,6,9-14	S P	NA		1-16	G P
α-GPDH	2,3,5,6,10-14	P P	2,3,5,6,10-15	P P	1-16	S S
G_2DH	2-6,8-14	G G	2-14	P P	1-16	P P
HK	5,9,10	P S	2-5,9-12	P P	1-16	S S
IDH	3-6,9-14	S G	3,5,6,8-13	S G	1-16	G G
LAP	5,6,9-14	G G	3,5,6,9,11,12	S G	1-16	G G
MDH	5-14	G S	3,5,6,8,9	P P	1-16	G G
ME	4,5,8,10-14	S S	NA		NA	
MR	2-16	S P	2-6,8,9,11,12	S S	1-16	S S
NADHDH	3,5,6,12	P P	5,6,10-12	P P	1-6	P P
NADPHDH	3,5,6,9-12	S S	3,5,6,9-12	P P	1-16	S P
PEP	2-5,10-12	S S	3-6,10-12	S S	1-16	G S
PER	1-16	S S	1-16	G S	1-16	S S
PC	3-6,10,11	G S	9-12	S S	1-16	G G
6PGD	4,5,9-11,14	G G	5,9-11	S P	1-16	G G
PGI	5,6,8-12,14	G P	5-7,11,12	G P	1-16	G S
PGM	4-6,10,11	G S	4-6,10,11	S S	1-16	G G
PPO	1-16	S S	1-16	S S	1-6	S S
SDH	6,8,10,12	S S	3,6,8,10,12	P P	1-16	G S
SUDH	9-14	P P	5,9-14	P P	1-16	P P
SOD	1-16	G S	1-16	S S	1-16	G S

Table 17. Number of isoenzyme bands observed for extracts prepared from black spruce needles using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	1	3	5	5	5	5	4	5	5	5	4	4	5	5	3	1
ACO	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0
AK	0	0	1	1	1	1	0	1	1	1	1	1	1	1	0	0
ADH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ALD	0	0	1	2	2	2	1	2	2	2	2	2	2	2	1	1
AAT	2	4	4	4	4	4	4	4	4	4	4	4	3	4	4	3
CTO	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
DIA	2	4	7	7	7	6	6	6	6	7	7	7	7	7	5	4
EST	0	2	3	3	3	3	3	3	3	3	3	3	3	3	3	2
F-EST	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
β-GAL	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
β-GLU	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GLUDH	0	0	2	1	1	1	0	0	0	0	1	2	2	1	0	0
G6PD	0	0	2	2	2	2	2	2	2	2	2	2	2	2	0	0
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
GPT	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
α-GPDH	0	2	2	0	2	2	0	0	0	2	2	2	2	2	0	0
G_2DH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HK	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
IDH	0	3	3	3	3	3	3	3	3	3	3	3	3	3	0	1
LAP	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2
MDH	0	5	4	4	4	4	4	4	4	4	4	4	4	4	2	2
ME	0	2	2	4	4	2	2	3	3	4	4	4	4	4	0	0
MR	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
NADHDH	0	0	2	0	2	1	0	1	1	1	2	2	1	1	0	0
NADPHDH	0	1	3	1	3	3	1	1	3	3	3	3	3	3	0	0
PEP	3	4	4	4	4	4	3	4	4	4	4	4	4	4	4	3
PER	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PC	0	1	1	1	1	1	0	1	1	1	1	1	0	1	0	0
6PGD	0	2	2	3	3	3	3	2	3	3	3	2	2	3	0	0
PGI	0	3	3	3	3	3	3	3	3	4	4	4	3	3	3	2
PGM	0	1	1	3	3	3	3	3	3	3	3	3	3	3	0	0
PPO	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
SDH	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0
SUDH	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0
SOD	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 18. Number of isoenzyme bands observed for extracts prepared from black spruce bark using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	4	4	5	5	5	5	5	5	5	5	5	5	5	5	4	3
ACO	0	1	1	1	1	1	0	1	0	0	1	1	1	1	0	0
AK	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ADH	0	0	0	0	2	2	0	2	2	2	2	2	2	2	2	0
ALD	0	1	1	2	2	2	2	2	2	2	2	2	2	2	2	1
AAT	2	3	4	4	4	4	4	4	4	4	4	4	2	4	4	0
CTO	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
DIA	3	4	5	6	6	5	5	6	6	7	7	7	6	6	3	2
EST	2	2	4	4	3	4	4	5	5	5	5	5	5	5	4	2
F-EST	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	1
β-GAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-GLU	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
GLUDH	0	0	2	1	1	1	1	1	1	2	2	2	2	2	0	0
G6PD	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-GPDH	0	2	2	0	2	2	0	0	0	2	2	2	2	2	2	0
G_2DH	0	2	2	2	2	2	2	2	2	2	2	2	2	2	0	0
HK	0	1	1	1	1	0	0	0	1	1	1	1	0	0	0	0
IDH	1	1	3	1	3	3	1	3	3	3	3	3	3	1	1	1
LAP	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1
MDH	2	3	4	4	3	4	3	3	3	4	4	4	4	4	4	3
ME	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MR	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
NADHDH	0	0	0	0	1	1	0	0	0	1	1	1	0	1	0	0
NADPHDH	0	1	2	1	2	2	1	1	2	2	2	2	1	1	1	0
PEP	0	2	4	4	4	4	3	4	4	4	4	4	4	4	4	1
PER	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
PC	0	0	1	1	1	1	0	0	1	1	1	1	0	1	0	0
6PGD	0	2	3	3	3	3	3	3	3	3	3	3	3	3	2	2
PGI	3	4	4	6	7	6	6	6	7	7	7	7	5	6	5	5
PGM	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0
PPO	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
SDH	0	2	2	2	2	2	2	2	2	2	2	2	2	2	0	0
SUDH	0	0	0	0	2	0	0	0	2	2	2	2	2	2	0	0
SOD	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Table 19. Number of isoenzyme bands observed for extracts prepared from black spruce buds using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	5	5	5	4	4	4	4	4	4	5	5	5	5	5	5	5
ACO	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AK	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
ADH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
ALD	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
AAT	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
CTO	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
DIA	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
EST	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
F-EST	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
β-GAL	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
β-GLU	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
GLUDH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G6PD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
α-GPDH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G_2DH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
HK	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
IDH	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
LAP	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
MDH	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
ME	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MR	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
NADHDH	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2
NADPHDH	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PEP	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PER	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PC	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6PGD	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PGI	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
PGM	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
PPO	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
SDH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3
SUDH	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SOD	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Table 20. Summary for tamarack of the best extraction buffers to use for each tissue and enzyme, and the type of activity and resolution observed (Act.=activity; Resol.=resolution; P=poor; S=satisfactory; G=good; NA=no activity)

ENZYME	NEEDLES			BARK			BUDS		
	Extraction Buffer No.	Act.	Resol.	Extraction Buffer No.	Act.	Resol.	Extraction Buffer No.	Act.	Resol.
ACP	2-5,9,11,12,14 15	G	S	2-5,8,9,15	S	P	1-16	G	S
ACO	NA			NA			NA		
AK	NA			2-15	P	P	9-11	G	S
ADH	NA			NA			NA		
ALD	NA			6,8,10-13	S	S	3-6,10,11	S	S
ALP	NA			NA			NA		
AAT	2-6,9,12	S	S	3,5,6,12,14	G	S	1-3,5-10	G	G
CAT	NA			NA			NA		
CTO	1-3	P	P	1-16	G	S	1-6	P	P
DIA	2-15	P	P	2-15	S	P	3-6,8-15	G	S
EST	3,9-11,15	S	P	2-5,9-11,14,15	S	P	1-16	S	P
F-EST	NA			NA			1-16	G	G
ß-GAL	NA			NA			NA		
ß-GLU	NA			4-14	S	P	1-16	G	P
GLUDH	9-12	P	P	3-14	P	P	5-15	S	P
G6PD	10,11	S	S	9-11	G	S	2-4,6,7,9-11,13	G	S
GDH	6,8,10-13	G	G	6,8	S	S	2-16	G	G
GPT	NA			9-11	G	P	1-16	G	P
G_3PDH	NA			NA			NA		
α-GPDH	NA			NA			NA		
G_2DH	NA			NA			10,11	S	S
HK	NA			3-14	P	P	9-11	G	S
IDH	3,4,9,10	S	S	4,6,8-10,13,14	G	G	1-16	G	G
LAP	NA			6,8-11	P	P	4-7	G	G
MDH	3-5,8-12	G	P	3-6,8,10-14	G	P	1-16	G	G
ME	NA			5,9-11	P	P	1-16	P	S
MR	3,4,9,10,15	P	P	4-6,8,9	S	P	1-16	G	S
NADHDH	NA			NA			1-16	P	P
NADPHDH	3-5,8-12,14	G	S	3-5,8-11	P	P	6,8,9	S	S
PEP	10,11	P	P	2,5,8-15	S	S	2,5-7,9-11,14	S	S
PER	1-16	S	S	1-16	G	G	1-16	S	S
PC	NA			NA			10-15	P	S
6PGD	3,4,6,9-11	P	P	4-6,9-12,14	G	S	1-16	G	G
PGI	1-3,9,10	P	P	2-14	G	P	1-16	G	P
PGM	3-5,8-10	P	S	4-6,8-11,14	G	S	1-16	G	G
PPO	1-10	P	P	1-16	S	S	1-16	P	P
SDH	3-5,9,10,12	P	P	3,5,8	P	P	4-8,10,11,13	G	G
SUDH	NA			NA			NA		
SOD	NA			1-16	S	S	1-16	P	P

Table 21. Number of isoenzyme bands observed for extracts prepared from tamarack needles using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	4	7	7	7	7	4	4	7	7	7	7	8	4	4	4	4
ACO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AK	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ADH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ALD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ALP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AAT	0	3	3	3	2	2	1	0	3	1	0	3	1	1	1	0
CAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CTO	3	3	3	1	1	1	1	1	1	1	1	1	1	1	1	1
DIA	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0
EST	0	7	7	7	7	7	7	7	7	7	7	7	7	7	7	0
F-EST	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-GAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-GLU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLUDH	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
G6PD	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
GDH	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G_3 PDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-GPDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G_2 DH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HK	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LAP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MDH	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2
ME	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MR	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	0
NADHDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NADPHDH	0	1	2	2	2	1	1	2	2	2	2	2	1	2	0	0
PEP	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
PER	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
PC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6PGD	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0
PGI	2	2	2	0	0	0	0	0	2	2	0	0	0	0	0	0
PGM	0	2	2	2	2	2	2	2	2	2	2	2	2	2	0	0
PPO	2	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0
SDH	0	0	2	2	2	0	0	0	2	2	0	2	0	0	0	0
SUDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 22. Number of isoenzyme bands observed for extracts prepared from tamarack bark using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	1	4	4	4	4	4	4	7	7	4	4	4	4	4	4	2
ACO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AK	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0
ADH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ALD	0	0	1	1	1	1	1	1	1	2	1	1	1	1	1	1
ALP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AAT	0	2	3	2	3	3	3	2	3	3	2	3	2	3	3	2
CAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CTO	7	7	6	7	7	7	7	7	7	6	6	6	6	4	4	4
DIA	0	4	4	4	4	5	4	5	4	4	4	5	5	4	4	4
EST	3	7	7	7	7	7	3	3	7	7	6	3	3	3	3	3
F-EST	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-GAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-GLU	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GLUDH	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0
G6PD	0	1	1	2	2	2	1	1	2	2	2	1	1	2	0	0
GDH	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
GPT	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
G_3 PDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-GPDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G_2 DH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HK	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0
IDH	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
LAP	0	0	1	1	2	2	1	2	2	2	2	2	1	2	1	0
MDH	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
ME	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0
MR	0	3	3	3	4	2	2	3	3	4	3	3	3	4	4	0
NADHDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NADPHDH	0	0	1	1	1	0	0	1	1	1	1	0	0	0	0	0
PEP	0	3	1	3	3	0	0	3	3	3	3	3	3	3	3	0
PER	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5
PC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6PGD	2	2	2	3	2	2	2	2	2	2	2	2	2	2	1	0
PGI	4	4	4	4	4	4	4	4	4	4	2	2	2	4	4	2
PGM	0	2	2	4	4	2	2	4	4	4	4	4	4	4	0	0
PPO	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SDH	0	2	2	2	2	3	2	2	2	2	2	2	2	2	0	0
SUDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Table 23. Number of isoenzyme bands observed for extracts prepared from tamarack buds using each of the 16 different extraction buffers

	EXTRACTION BUFFER NO.															
ENZYME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ACP	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
ACO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AK	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
ADH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ALD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
ALP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AAT	6	6	6	4	6	6	6	6	6	6	6	6	6	6	6	6
CAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CTO	5	5	5	5	5	5	3	3	3	3	3	3	3	3	3	3
DIA	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
EST	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
F-EST	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
β-GAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-GLU	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GLUDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G6PD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
GDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GPT	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G_3 PDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-GPDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G_2 DH	2	2	2	2	2	2	2	2	2	2	2	2	2	0	0	0
HK	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
IDH	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	2
LAP	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
MDH	4	4	4	6	4	6	6	6	6	6	4	4	4	4	4	4
ME	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MR	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
NADHDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
NADPHDH	2	2	2	3	3	3	3	3	3	3	3	1	1	1	1	1
PEP	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
PER	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
PC	0	0	1	1	1	1	0	1	1	1	1	1	1	1	0	0
6PGD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PGI	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PGM	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PPO	1	1	1	1	1	1	1	2	2	2	2	2	1	1	1	1
SDH	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
SUDH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Table 24. **Summary of the number of times an extraction buffer was listed for the various enzymes analysed, as shown in Tables 4, 8, 12, 16 and 20. The total number of enzymes examined for each species are: jack pine, 41; Scots pine, 36; white spruce, 40; black spruce, 37; and, tamarack, 40 (N, Needles; B, Bark)**

Extraction buffer No.	NUMBER OF TIMES EXTRACTION BUFFER LISTED FOR ENZYMES ANALYZED														
	Jack pine			Scots pine			White spruce			Black spruce			Tamarack		
	N	B	BUD	N	B	BUD	N	B	BUD	N	B	BUD	N	B	BUD
1	4	11	12	6	6	9	6	5	27	5	6	33	5	5	19
2	16	20	29	15	11	17	18	16	29	10	11	34	8	11	22
3	33	20	29	13	11	17	22	28	33	19	20	35	15	16	23
4	22	18	31	7	8	15	21	26	38	21	15	35	12	19	23
5	23	27	36	19	18	26	29	33	39	30	23	36	9	22	25
6	29	23	29	15	15	24	22	22	36	28	22	35	6	20	27
7	6	15	23	12	11	22	8	13	30	9	8	30	3	11	23
8	21	16	24	12	12	22	15	15	31	17	12	30	7	22	22
9	24	26	35	13	15	29	28	22	32	23	20	31	15	23	25
10	29	27	27	19	19	30	28	29	32	32	28	31	16	23	28
11	23	25	34	25	24	32	23	29	33	31	29	32	11	22	27
12	26	27	26	30	28	32	23	22	29	30	28	32	9	16	20
13	16	20	24	24	18	31	19	20	30	20	14	31	3	15	22
14	16	22	28	25	21	28	17	22	33	24	14	31	4	18	21
15	7	11	18	13	8	18	7	11	29	7	9	30	5	10	20
16	4	9	16	7	6	8	4	4	24	6	6	29	1	5	17